PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number: WO 93/19179
C12N 15/12, C07K 13/00 C12P 21/08, C12N 15/11, 9/00 C12N 1/21, 5/10, G01N 33/68	A1	(43) International Publication Date: 30 September 1993 (30.09.93)

(21) International Application Number: PCT/US93/02569

(22) International Filing Date: 19 March 1993 (19.03.93)

(30) Priority data:

07/854,296	19 March 1992 (19.03.92)	US
07/980,498	23 November 1992 (23.11.92)	US

(71) Applicant: THE ROCKEFELLER UNIVERSITY [US/US]; 1230 York Avenue, New York, NY 10021 (US).

(72) Inventors: DARNELL, James, E., Jr. ; 22 Chestnut Street, Larchmont, NY 10538 (US). SCHINDLER, Christian, W. ; 18 Terrell Avenue, Rockville Center, New York, NY 11570 (US). FU, Xin-Yuan ; 1249 Park Avenue, Apt. 2A, New York, NY 10029 (US). SHUAI, Ke ; 500 East 63rd Street, Apt. 22D, New York, NY 10021 (US).

(74) Agent: JACKSON, David, A.; Klauber & Jackson, 411 Hackensack Avenue, Hackensack, NJ 07601 (US).

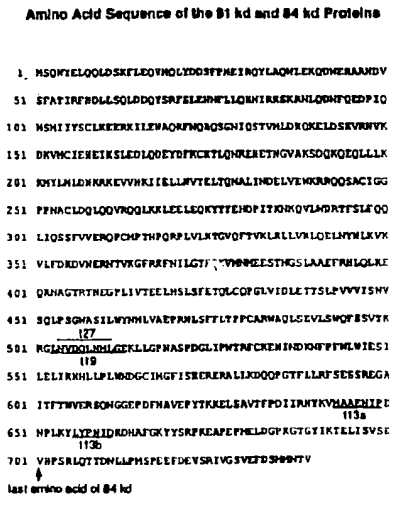
(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: IFN RECEPTORS RECOGNITION FACTORS, PROTEIN SEQUENCES AND METHODS OF USE THEREOF

(57) Abstract

Receptor recognition factors exist that recognize the specific cell receptor to which a specific ligand has been bound, and that may thereby signal and/or initiate the binding of the transcription factor to the DNA site. The receptor recognition factor is in one instance, a part of a transcription factor, and also may interact with other transcription factors to cause them to activate and travel to the nucleus for DNA binding. The receptor recognition factor appears to be second-messenger-independent in its activity, as overt perturbations in second messenger concentrations are of no effect. The concept of the invention is illustrated by the results of studies conducted with interferon (IFN)-stimulated gene transcription, and particularly, the activation caused by both IFNα and IFNγ. Specific DNA sequences have been prepared that correspond to polypeptide fragments of two of the ISGF-3 genes, and antibodies have also been prepared and tested. The polypeptides confirm direct involvement of tyrosine kinase in intracellular message transmission. Numerous diagnostic and therapeutic materials and utilities are also disclosed.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**FR**	France	**MR**	Mauritania
AU	Australia	**GA**	Gabon	**MW**	Malawi
BB	Barbados	**GB**	United Kingdom	**NL**	Netherlands
BE	Belgium	**GN**	Guinea	**NO**	Norway
BF	Burkina Faso	**GR**	Greece	**NZ**	New Zealand
BG	Bulgaria	**HU**	Hungary	**PL**	Poland
BJ	Benin	**IE**	Ireland	**PT**	Portugal
BR	Brazil	**IT**	Italy	**RO**	Romania
CA	Canada	**JP**	Japan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SK**	Slovak Republic
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SN**	Senegal
CM	Cameroon	**LI**	Liechtenstein	**SU**	Soviet Union
CS	Czechoslovakia	**LK**	Sri Lanka	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**MC**	Monaco	**UA**	Ukraine
DK	Denmark	**MG**	Madagascar	**US**	United States of America
ES	Spain	**ML**	Mali	**VN**	Viet Nam
FI	Finland	**MN**	Mongolia		

IFN RECEPTORS RECOGNITION FACTORS, PROTEIN SEQUENCES AND METHODS OF USE THEREOF.

RELATED PUBLICATIONS

The Applicants are authors or co-authors of several articles directed to the subject matter of the present invention. (1) Darnell et al.,"Interferon-Dependent Transcriptional Activation: Signal Transduction Without Second Messenger Involvement?" THE NEW BIOLOGIST, 2(10):1-4, (1990); (2) X. Fu et al., "ISGF3, The Transcriptional Activator Induced by Interferon α, Consists of Multiple Interacting Polypeptide Chains" PROC. NATL. ACAD. SCI. USA, 87:8555-8559 (1990); (3) D.S. Kessler et al., "IFNα Regulates Nuclear Translocation and DNA-Binding Affinity of ISGF3, A Multimeric Transcriptional Activator" GENES AND DEVELOPMENT, 4:1753 (1990). All of the above listed articles are incorporated herein by reference.

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to intracellular receptor recognition proteins or factors(i.e. groups of proteins), and to methods and compositions including such factors or the antibodies reactive toward them, or analogs thereof in assays and for diagnosing, preventing and/or treating cellular debilitation, derangement or dysfunction. More particularly, the present invention relates to particular IFN-dependent receptor recognition molecules that have been identified and sequenced, and that demonstrate direct participation in intracellular events, extending from interaction with the liganded receptor at the cell surface to transcription in the nucleus, and to antibodies or to other entities specific thereto that may thereby selectively modulate such activity in mammalian cells.

BACKGROUND OF THE INVENTION

There are several possible pathways of signal transduction that might be followed after a polypeptide ligand binds to its cognate cell surface receptor. Within

minutes of such ligand-receptor interaction, genes that were previously quiescent are rapidly transcribed (Murdoch et al., 1982; Larner et al., 1984; Friedman et al., 1984; Greenberg and Ziff, 1984; Greenberg et al., 1985). One of the most physiologically important, yet poorly understood, aspects of these immediate transcriptional responses is their specificity: the set of genes activated, for example, by platelet-derived growth factor (PDGF), does not completely overlap with the one activated by nerve growth factor (NGF) or tumor necrosis factor (TNF) (Cochran et al., 1983; Greenberg et al., 1985; Almendral et al., 1988; Lee et al., 1990). The interferons (IFN) activate sets of other genes entirely. Even IFNα and IFNγ, whose presence results in the slowing of cell growth and in an increased resistance to viruses (Tamm et al., 1987) do not activate exactly the same set of genes (Larner et al., 1984; Friedman et al., 1984; Celis et al., 1987, 1985; Larner et al., 1986).

The current hypotheses related to signal transduction pathways in the cytoplasm do not adequately explain the high degree of specificity observed in polypeptide-dependent transcriptional responses. The most commonly discussed pathways of signal transduction that might ultimately lead to the nucleus depend on properties of cell surface receptors containing tyrosine kinase domains [for example, PDGF, epidermal growth factor (EGF), colony-stimulating factor (CSF), insulin-like growth factor-1 (IGF-1); see Gill, 1990; Hunter, 1990) or of receptors that interact with G-proteins (Gilman, 1987). These two groups of receptors mediate changes in the intracellular concentrations of second messengers that, in turn, activate one of a series of protein phosphokinases, resulting in a cascade of phosphorylations (or dephosphorylations) of cytoplasmic proteins.

It has been widely conjectured that the cascade of phosphorylations secondary to changes in intracellular second messenger levels is responsible for variations in the rates of transcription of particular genes (Bourne, 1988, 1990; Berridge, 1987; Gill, 1990; Hunter, 1990). However, there are at least two reasons to question the suggestion that global changes in second messengers participate in the chain of

events leading to specific transcriptional responses dependent on specific receptor occupation by polypeptide ligands.

First, there is a limited number of second messengers (cAMP, diacyl glycerol, phosphoinositides, and Ca^{2+} are the most prominently discussed), whereas the number of known cell surface receptor-ligand pairs of only the tyrosine kinase and G-protein varieties, for example, already greatly outnumbers the list of second messengers, and could easily stretch into the hundreds (Gill, 1990; Hunter, 1990). In addition, since many different receptors can coexist on one cell type at any instant, a cell can be called upon to respond simultaneously to two or more different ligands with an individually specific transcriptional response each involving a different set of target genes. Second, a number of receptors for polypeptide ligands are now known that have neither tyrosine kinase domains nor any structure suggesting interaction with G-proteins. These include the receptors for interleukin-2 (IL-2) (Leonard et al., 1985), IFNα (Uze et al., 1990), IFNγ (Aguet et al., 1988), NGF (Johnson et al., 1986), and growth hormone (Leung et al., 1987). The binding of each of these receptors to its specific ligand has been demonstrated to stimulate transcription of a specific set of genes. For these reasons it seems unlikely that global intracellular fluctuations in a limited set of second messengers are integral to the pathway of specific, polypeptide ligand-dependent, immediate transcriptional responses.

In PCT International Publication No. WO 92/08740 published 29 May, 1992 by the applicant herein, the above analysis was presented and it was discovered and proposed that a receptor recognition factor or factors, served in some capacity as a type of direct messenger between liganded receptors at the cell surface and the cell nucleus. One of the characteristics that was ascribed to the receptor recognition factor was its apparent lack of requirement for changes in second messenger concentrations. Continued investigation of the receptor recognition factor through study of the actions of the interferons IFNα and IFNγ has further elucidated the characteristics and structure of the interferon-related factor ISGF-3, and more

broadly, the characterization and structure of the receptor recognition factor in a manner that extends beyond earlier discoveries previously described. It is accordingly to the presentation of this updated characterization of the receptor recognition factor and the materials and methods both diagnostic and therapeutic corresponding thereto that the present disclosure is directed.

SUMMARY OF THE INVENTION

In accordance with the present invention, receptor recognition factors have been further characterized that appear to interact directly with receptors that have been occupied by their ligand on cellular surfaces, and which in turn either become active transcription factors, or activate or directly associate with transcription factors that enter the cells' nucleus and specifically binds on predetermined sites and thereby activates the genes. It should be noted that the receptor recognition proteins thus possess multiple properties, among them: 1) recognizing and being activated during such recognition by receptors; 2) being translocated to the nucleus by an inhibitable process (eg. NaF inhibits translocation); and 3) combining with transcription activating proteins or acting themselves as transcription activation proteins, and that all of these properties are possessed by the proteins described herein.

The receptor recognition factor is proteinaceous in composition and is believed to be present in the cytoplasm. The recognition factor is not demonstrably affected by concentrations of second messengers, however does exhibit direct interaction with tyrosine kinase domains, although it exhibits no apparent interaction with G-proteins. More particularly, the factor represented by SEQ ID NO:2 directly interacts with DNA after acquiring phosphate on tyrosine located at or about position 690 of the amino acid sequence.

The recognition factor is now known to comprise several proteinaceous substituents, in the instance of IFNα and IFNγ. Particularly, three proteins

derived from the factor ISGF-3 have been successfully sequenced and their sequences are set forth in FIGURES 1, 2 and 3 herein, and corresponding Sequence Identification Nos. 1, 2 and 3. It is particularly noteworthy that the protein sequence of FIGURE 1 and the sequence of the proteins of FIGURES 2 and 3 derive, respectively, from two different but related genes. It is clear from this discovery that a family of genes exists, and that further family members likewise exist. Accordingly, by use of hybridization techniques, additional such family members will be found. Further, the capacity of such family members to function in the manner of the receptor recognition factors disclosed, herein may be assessed by determining those ligand that cause the phosphorylation of the particular family members.

In its broadest aspect, the present invention extends to a receptor recognition factor implicated in the transcriptional stimulation of genes in target cells in response to the binding of a specific polypeptide ligand to its cellular receptor on said target cell, said receptor recognition factor having the following characteristics:

a) apparent direct interaction with the ligand-bound receptor complex and activation of one or more transcription factors capable of binding with a specific gene;

b) an activity demonstrably unaffected by the presence or concentration of second messengers;

c) direct interaction with tyrosine kinase domains; and

d) a perceived absence of interaction with G-proteins.

More particularly, the receptor recognition factor represented by SEQ ID NO:2 possesses the added capability of acting as a translation protein and, in particular, as a DNA binding protein in response to interferon-γ stimulation. This discovery presages an expanded role for the proteins in question, and other proteins and like factors that have heretofore been characterized as receptor recognition factors. It is therefore apparent that a single factor may indeed provide the nexus between the

liganded receptor at the cell surface and direct participation in DNA transcriptional activity in the nucleus. This pleiotypic factor has the following characteristics:

a) It interacts with an interferon-γ-bound receptor kinase complex;

b) It is a tyrosine kinase substrate; and

c) When phosphorylated, it serves as a DNA binding protein.

More particularly, the factor represented by SEQ ID NO:2 is interferon-dependent in its activity and is responsive to interferon stimulation, particularly that of interferon-γ.

In a still further aspect, the present invention extends to a receptor recognition factor interactive with a liganded interferon receptor, which receptor recognition factor possesses the following characteristics:

a) it is present in cytoplasm;

b) it undergoes tyrosine phosphorylation upon treatment of cells with IFNα or IFNγ;

c) it activates transcription of an interferon stimulated gene;

d) it stimulates either an ISRE-dependent or a gamma activated site (GAS)-dependent transcription *in vivo*;

e) it interacts with IFN cellular receptors, and

f) it undergoes nuclear translocation upon stimulation of the IFN cellular receptors with IFN.

The factor of the invention represented by SEQ ID NO:2 appears to act in similar fashion to an earlier determined site-specific DNA binding protein that is interferon-γ dependent and that has been earlier called the γ activating factor (GAF). Specifically, interferon-γ-dependent activation of this factor occurs without new protein synthesis and appears within minutes of interferon-γ treatment, achieves maximum extent between 15 and 30 minutes thereafter, and then disappears after 2-3 hours. These further characteristics of identification and

action assist in the evaluation of the present factor for applications having both diagnostic and therapeutic significance.

The present invention also relates to a recombinant DNA molecule or cloned gene, or a degenerate variant thereof, which encodes a receptor recognition factor, or a fragment thereof, that possesses a molecular weight and DNA sequence selected from a molecular weight of about 113 kD and the DNA sequence set forth in FIGURE 1 (SEQ ID NO:1), a molecular weight of about 91 kD and the DNA sequence set forth in FIGURE 2 (SEQ ID NO:2), and a molecular weight of about 84 kD and the DNA sequence set forth in FIGURE 3 (SEQ ID NO:3).

The human DNA sequences of the receptor recognition factors of the present invention or portions thereof, may be prepared as probes to screen for complementary sequences and genomic clones in the same or alternate species. The present invention extends to probes so prepared that may be provided for screening cDNA and genomic libraries for the receptor recognition factors. For example, the probes may be prepared with a variety of known vectors, such as the phage λ vector. The present invention also includes the preparation of plasmids including such vectors, and the use of the DNA sequences to construct vectors expressing antisense RNA or ribozymes which would attack the mRNAs of any or all of the DNA sequences set forth in FIGURES 1, 2 and 3. Correspondingly, the preparation of antisense RNA and ribozymes are included herein.

The present invention also includes receptor recognition factor proteins having the activities noted herein, and that display the amino acid sequences set forth and described above and selected from SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

In a further embodiment of the invention, the full DNA sequence of the recombinant DNA molecule or cloned gene so determined may be operatively linked to an expression control sequence which may be introduced into an

appropriate host. The invention accordingly extends to unicellular hosts transformed with the cloned gene or recombinant DNA molecule comprising a DNA sequence encoding the present receptor recognition factor(s), and more particularly, the complete DNA sequence determined from the sequences set forth above and in SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

According to other preferred features of certain preferred embodiments of the present invention, a recombinant expression system is provided to produce biologically active animal or human receptor recognition factor.

The concept of the receptor recognition factor contemplates that specific factors exist for correspondingly specific ligands, such as tumor necrosis factor, nerve growth factor and the like, as described earlier. Accordingly, the exact structure of each receptor recognition factor will understandably vary so as to achieve this ligand and activity specificity. It is this specificity and the direct involvement of the receptor recognition factor in the chain of events leading to gene activation, that offers the promise of a broad spectrum of diagnostic and therapeutic utilities.

The present invention naturally contemplates several means for preparation of the recognition factor, including as illustrated herein known recombinant techniques, and the invention is accordingly intended to cover such synthetic preparations within its scope. The isolation of the cDNA amino acid sequences disclosed herein facilitates the reproduction of the recognition factor by such recombinant techniques, and accordingly, the invention extends to expression vectors prepared from the disclosed DNA sequences for expression in host systems by recombinant DNA techniques, and to the resulting transformed hosts.

The invention includes an assay system for screening of potential drugs effective to modulate transcriptional activity of target mammalian cells by interrupting or potentiating the recognition factor or factors. In one instance, the test drug could be administered to a cellular sample with the ligand that activates the receptor

recognition factor, or an extract containing the activated recognition factor, to determine its effect upon the binding activity of the recognition factor to any chemical sample (including DNA), or to the test drug, by comparison with a control.

The assay system could more importantly be adapted to identify drugs or other entities that are capable of binding to the receptor recognition and/or transcription factors or proteins, either in the cytoplasm or in the nucleus, thereby inhibiting or potentiating transcriptional activity. Such assay would be useful in the development of drugs that would be specific against particular cellular activity, or that would potentiate such activity, in time or in level of activity. For example, such drugs might be used to modulate cellular response to shock, or to treat other pathologies, as for example, in making IFN more potent against cancer.

One of the characteristics of the present receptor recognition factors is their participation in rapid phosphorylation and dephosphorylation during the course of and as part of their activity. Significantly, such phosphorylation takes place in an interferon-dependent manner and within a few minutes in the case of the ISGF-3 proteins identified herein, on the tyrosine residues defined thereon. This is strong evidence that the receptor recognition factors disclosed herein are the first true substrates whose intracellular function is well understood and whose intracellular activity depends on tyrosine kinase phosphorylation. In particular, the addition of phosphate to the tyrosine of a transcription factor is novel. This suggests further that tyrosine kinase takes direct action in the transmission of intracellular signals to the nucleus, and does not merely serve as a promoter or mediator of serine and/or serinine kinase activity, as has been theorized to date. Also, the role of the factor represented by SEQ ID NO:2 in its activated phosphorylated form suggests possible independent therapeutic use for this activated form. Likewise, the role of the factor as a tyrosine kinase substrate suggests its interaction with kinase in other theatres apart from the complex observed herein.

The diagnostic utility of the present invention extends to the use of the present receptor recognition factors in assays to screen for tyrosine kinase inhibitors. Because the activity of the receptor recognition-transcriptional activation proteins described herein must maintain tyrosine phosphorylation, they can and presumably are dephosphorylated by specific tyrosine phosphatases. Blocking of the specific phosphatase is therefore an avenue of pharmacological intervention that would potentiate the activity of the receptor recognition proteins.

The present invention likewise extends to the development of antibodies against the receptor recognition factor(s), including naturally raised and recombinantly prepared antibodies. For example, the antibodies could be used to screen expression libraries to obtain the gene or genes that encode the receptor recognition factor(s). Such antibodies could include both polyclonal and monoclonal antibodies prepared by known genetic techniques, as well as bi-specific (chimeric) antibodies, and antibodies including other functionalities suiting them for additional diagnostic use conjunctive with their capability of modulating transcriptional activity.

In particular, antibodies against specifically phosphorylated factors can be selected and are included within the scope of the present invention for their particular ability in following activated protein. Thus, activity of the recognition factors or of the specific polypeptides believed to be causally connected thereto may therefore be followed directly by the assay techniques discussed later on, through the use of an appropriately labeled quantity of the recognition factor or antibodies or analogs thereof.

Thus, the receptor recognition factors, their analogs and/or analogs, and any antagonists or antibodies that may be raised thereto, are capable of use in connection with various diagnostic techniques, including immunoassays, such as a radioimmunoassay, using for example, an antibody to the receptor recognition

factor that has been labeled by either radioactive addition, reduction with sodium borohydride, or radioiodination.

In an immunoassay, a control quantity of the antagonists or antibodies thereto, or the like may be prepared and labeled with an enzyme, a specific binding partner and/or a radioactive element, and may then be introduced into a cellular sample. After the labeled material or its binding partner(s) has had an opportunity to react with sites within the sample, the resulting mass may be examined by known techniques, which may vary with the nature of the label attached. For example, antibodies against specifically phosphorylated factors may be selected and appropriately employed in the exemplary assay protocol, for the purpose of following activated protein as described above.

In the instance where a radioactive label, such as the isotopes ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re are used, known currently available counting procedures may be utilized. In the instance where the label is an enzyme, detection may be accomplished by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques known in the art.

The present invention includes an assay system which may be prepared in the form of a test kit for the quantitative analysis of the extent of the presence of the recognition factors, or to identify drugs or other agents that may mimic or block their activity. The system or test kit may comprise a labeled component prepared by one of the radioactive and/or enzymatic techniques discussed herein, coupling a label to the recognition factors, their agonists and/or antagonists, and one or more additional immunochemical reagents, at least one of which is a free or immobilized ligand, capable either of binding with the labeled component, its binding partner, one of the components to be determined or their binding partner(s).

In a further embodiment, the present invention relates to certain therapeutic methods which would be based upon the activity of the recognition factor(s), its (or their) subunits, or active fragments thereof, or upon agents or other drugs determined to possess the same activity. A first therapeutic method is associated with the prevention of the manifestations of conditions causally related to or following from the binding activity of the recognition factor or its subunits, and comprises administering an agent capable of modulating the production and/or activity of the recognition factor or subunits thereof, either individually or in mixture with each other in an amount effective to prevent the development of those conditions in the host. For example, drugs or other binding partners to the receptor recognition/transcription factors or proteins may be administered to inhibit or potentiate transcriptional activity, as in the potentiation of interferon in cancer therapy. Also, the blockade of the action of specific tyrosine phosphatases in the dephosphorylation of activated (phosphorylated) recognition/transcription factors or proteins presents a method for potentiating the activity of the receptor recognition factor or protein that would concomitantly potentiate therapies based on receptor recognition factor/protein activation.

More specifically, the therapeutic method generally referred to herein could include the method for the treatment of various pathologies or other cellular dysfunctions and derangements by the administration of pharmaceutical compositions that may comprise effective inhibitors or enhancers of activation of the recognition factor or its subunits, or other equally effective drugs developed for instance by a drug screening assay prepared and used in accordance with a further aspect of the present invention. For example, drugs or other binding partners to the receptor recognition/transcription factor or proteins, as represented by SEQ ID NO:2, may be administered to inhibit or potentiate transcriptional activity, as in the potentiation of interferon in cancer therapy. Also, the blockade of the action of specific tyrosine phosphatases in the dephosphorylation of activated (phosphorylated) recognition/transcription factor or protein presents a method for potentiating the activity of the receptor recognition factor or protein

that would concomitantly potentiate therapies based on receptor recognition factor/protein activation. Correspondingly, the inhibition or blockade of the activation or binding of the recognition/transcription factor would affect MHC Class II expression and consequently, would promote immunosuppression. Materials exhibiting this activity, as illustrated later on herein by staurosporine, may be useful in instances such as the treatment of autoimmune diseases and graft rejection, where a degree of immunosuppression is desirable.

In particular, the proteins of ISGF-3 whose sequences are presented in SEQ ID NOS: 1-3 herein, their antibodies, agonists, antagonists, or active fragments thereof, could be prepared in pharmaceutical formulations for administration in instances wherein interferon therapy is appropriate, such as to treat chronic viral hepatitis, hairy cell leukemia, and for use of interferon in adjuvant therapy. The specificity of the receptor proteins hereof would make it possible to better manage the aftereffects of current interferon therapy, and would thereby make it possible to apply interferon as a general antiviral agent.

Accordingly, it is a principal object of the present invention to provide a receptor recognition factor and its subunits in purified form that exhibits certain characteristics and activities associated with transcriptional promotion of cellular activity.

It is a further object of the present invention to provide antibodies to the receptor recognition factor and its subunits, and methods for their preparation, including recombinant means.

It is a further object of the present invention to provide a method for detecting the presence of the receptor recognition factor and its subunits in mammals in which invasive, spontaneous, or idiopathic pathological states are suspected to be present.

It is a further object of the present invention to provide a method and associated assay system for screening substances such as drugs, agents and the like, potentially effective in either mimicking the activity or combating the adverse effects of the recognition factor and/or its subunits in mammals.

It is a still further object of the present invention to provide a method for the treatment of mammals to control the amount or activity of the recognition factor or subunits thereof, so as to alter the adverse consequences of such presence or activity, or where beneficial, to enhance such activity.

It is a still further object of the present invention to provide a method for the treatment of mammals to control the amount or activity of the recognition factor or its subunits, so as to treat or avert the adverse consequences of invasive, spontaneous or idiopathic pathological states.

It is a still further object of the present invention to provide pharmaceutical compositions for use in therapeutic methods which comprise or are based upon the recognition factor, its subunits, their binding partner(s), or upon agents or drugs that control the production, or that mimic or antagonize the activities of the recognition factors.

Other objects and advantages will become apparent to those skilled in the art from a review of the ensuing description which proceeds with reference to the following illustrative drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 depicts the full receptor recognition factor nucleic acid sequence and the deduced amino acid sequence derived for the ISGF-3α gene defining the 113 kD protein. The nucleotides are numbered from 1 to 2553, and the amino acids are numbered from 1 to 851. This sequence is identically depicted in the

SEQUENCE LISTING presented later on herein, in accordance with 37 C.F.R. 1.821-825, enacted October 1, 1990, and is cumulatively and alternately referred to as SEQ ID NO:1.

FIGURE 2 depicts the full receptor recognition factor nucleic acid sequence and the deduced amino acid sequence derived for the ISGF-3α gene defining the 91 kD protein. The nucleotides are numbered from 1 to 2217, and the amino acids are numbered from 1 to 739. This sequence is identically depicted in the SEQUENCE LISTING presented later on herein, in accordance with 37 C.F.R. 1.821-825, enacted October 1, 1990, and is cumulatively and alternately referred to as SEQ ID NO:2.

FIGURE 3 depicts the full receptor recognition factor nucleic acid sequence and the deduced amino acid sequence derived for the ISGF-3α gene defining the 84 kD protein. The nucleotides are numbered from 1 to 2103, and the amino acids are numbered from 1 to 701. This sequence is identically depicted in the SEQUENCE LISTING presented later on herein, in accordance with 37 C.F.R. 1.821-825, enacted October 1, 1990, and is cumulatively and alternately referred to as SEQ ID NO:3.

FIGURE 4 shows the purification of ISGF-3. The left-hand portion of the Figure shows the purification of ISGF-3 demonstrating the polypeptides present after the first oligonucleotide affinity column (lane 3) and two different preparations after the final chromatography step (Lanes 1 and 2). The left most lane contains protein size markers (High molecular weight, Sigma). ISGF-3 component proteins are indicated as 113 kD, 91 kD, 84 kD, and 48 kD [Kessler et al., *GENES & DEV.*, **4** (1990); Levy et al., *THE EMBO. J.*, **9** (1990)]. The right-hand portion of the Figure shows purified ISGF-3 from 2-3 x 10^{11} cells was electroblotted to nitrocellulose after preparations 1 and 2 (Lanes 1 and 2) had been pooled and separated on a 7.5% SDS polyacrylamide gel. ISGF-3 component proteins are

indicated. The two lanes on the right represent protein markers (High molecular weight, and prestained markers, Sigma).

FIGURE 5 generally presents the results of Northern Blot analysis for the 91/84 kD peptides. Figure 5a presents restriction maps for cDNA clones E4 (top map) and E3 (bottom map) showing DNA fragments that were radiolabeled as probes (probes A-D). Figure 5b comprises Northern blots of cytoplasmic HeLa RNA hybridized with the indicated probes. The 4.4 and 3.1 KB species as well as the 28S and 18S rRNA bands are indicated.

FIGURE 6 depicts the conjoint protein sequence of the 91 kD and 84 kD proteins of ISGF-3. One letter amino acid code is shown for the open reading frame from clone E4, (encoding the 91 kD protein). The 84 kD protein, encoded by a different cDNA (E3), has the identical sequence but terminates after amino acid 701, as indicated. Tryptic peptides t19, t13a, and t13b from the 91 kD protein are indicated. The sole recovered tryptic peptide from the 84 kD protein, peptide t27, was wholly contained within peptide t19 as indicated.

FIGURE 7 presents the results of Western blot and antibody shift analyses.

a) Highly purified ISGF-3, fractionated on a 7.0% SDS polyacrylamide gel, was probed with antibodies a42 (amino acids 597-703); a55 (amino acids 2-59); and a57 (amino acids 705-739) in a Western blot analysis. The silver stained part of the gel (lanes a, b, and c) illustrates the location of the ISGF-3 component proteins and the purity of the material used in Western blot: Lane a) Silver stain of protein sample used in all the Western blot experiments (immune and preimmune). Lane b) Material of equal purity to that shown in Fig. 4, for clearer identification of the ISGF-3 proteins. Lane c) Size protein markers indicated.

b) Antibody interference of the ISGF-3 shift complex; Lane a) The complete ISGF-3 and the free ISGF-3γ component shift with partially purified ISGF-3 are marked; Lane b) Competition with a 100 fold excess of cold ISRE

oligonucleotide. Lane c) Shift complex after the addition of 1 ml of preimmune serum to a 12.5 μl shift reaction. Lanes d and e) - Shift complex after the addition of 1 μl of a 1:10 dilution or 1 ml of undiluted a42 antiserum to a 12.5 μl shift reaction.

Methods:

Antibodies a42, a55 and a57 were prepared by injecting approximately 500 mgm of a fusion protein prepared in E. coli using the GE3-3X vector [Smith et al., *GENE*, **67** (1988)]. Rabbits were bled after the second boost and serum prepared.

For Western blots highly purified ISGF-3 was separated on a 7% SDS polyacrylamide gel and electroblotted to nitrocellulose. The filter was incubated in blocking buffer ("blotto"), cut into strips and probed with specific antiserum and preimmune antiserum diluted 1:500. The immune complexes were visualized with the aid of an ECL kit (Amersham). Shift analyses were performed as previously described [Levy et al., *GENES & DEV.*, **2** (1988); Levy et al., *GENES & DEV.*, **3** (1989)] in a 4.5% polyacrylamide gel.

FIGURE 8 presents the full length amino acid sequence of 113 kD protein components of ISGF-3α and alignment of conserved amino acid sequences between the 113 kD and 91/84 kD proteins.

A. Polypeptide sequences (A-E) derived from protein micro-sequencing of purified 113 kD protein (see accompanying paper) are underlined. Based on peptide E, we designed a degenerate oligonucleotide, AAT/CACIGAA/GCCIATGGAA/GATT/CATT, which was used to screen a cDNA library [Pine et la., *MOL. CELL. BIOL.*, **10** (1990)] basically as described [Norman et al., *CELL*, **55** (1988)]. Briefly, the degenerate oligonucleotides were labeled by 32P-γ-ATP by polynucleotide kinase, hybridizations were carried out overnight at 40°C in 6 x SSTE (0.9 M NaCl, 60 mM Tris-HCl [pH 7.9] 6mM EDTA), 0.1%SDS, 2mM $Na_2P_5O_7$, 6 mM KH_2PO_4 in the presence of 100 mg/ml salmon sperm DNA sperm and 10 x Denhardt's solution [Maniatis et al.,

MOLECULAR CLONING; A LABORATORY MANUAL (Cold Spring Harbor Lab., 1982)]. The nitrocellulose filters then were washed 4 x 10 min. with the same hybridization conditions without labeled probe and salmon sperm DNA. Autoradiography was carried out at -80°C with intensifying screen for 48 hrs. A PCR product was obtained later by the same method described for the 91/84 kD sequences, by using oligonucleotides designed according polypeptide D and E: The sequence of this PCR product was identical to a region in clone f11. The full length of 113 kD protein contains 851 amino acids. Three major helices in the N-terminal region were predicted by the methods of both Chou and Fasman [Chou et al., *ANN. REV. BIOCHEM.*, **47** (1978)] and Garnier et al [Garnier et al., *J. MOL. BIOL.*, **12** (1978)] and are shown in shadowed boxes. At the C-terminal end, a highly negative charged domain was found. All negative charged residues are blackened and positive charged residues shadowed. The five polypeptides that derived from protein microscreening [Aebersold et al., *PROC. NATL. ACAD. SCI. USA*, **87** (1987)] are underlined.

B) Comparison of amino acid sequences of 113 kD and 91/84 kD protein shows a 42% identical amino acid residues in the overlapping 715 amino acid sequence shown. In the middle helix region four leucine and one valine heptad repeats were identified in both 113 and 91/84 kD protein (the last leucine in 91/84 kD is not exactly preserved as heptad repeats). When a heligram structure was drawn this helix is amphipathic (not shown). Another notable feature of this comparison is several tyrosine residues that are conserved in both proteins near their ends.

FIGURE 9 shows the *in vitro* transcription and translation of 113 kD and 91 kD cDNA and a Northern blot analysis with 113 kD cDNA probe.

a) The full length cDNA clones of 113 and 91 kD protein were transcribed *in vitro* and transcribed RNAs was translated *in vitro* with rabbit lenticulate lysate (Promega; conditions as described in the Promega protocol). The mRNA of BMV (Promega) was simultaneously translated as a protein size

marker. The 113 cDNA yielded a translated product about 105 kD and the 91 cDNA yielded a 86 kD product.

b) When total cytoplasmic mRNAs isolated from superinduced HeLa cells were utilized, a single 4.8 KB mRNA band was observed with a cDNA probe coding for C-end of 113 kD protein in a Northern blot analysis [Nielsch et al., *The EMBO. J.*, **10** (1991)].

FIGURE 10(A) presents the results of Western blot analysis confirming the identity of the 113 kD protein. An antiserum raised against a polypeptide segment [Harlow et al., *ANTIBODIES; A LABORATORY MANUAL* (Cold Spring Harbor Lab., 1988)] from amino acid 500 to 650 of 113 kD protein recognized specifically a 113 kD protein in a protein Western blot analysis. The antiserum recognized a band both in a highly purified ISGF-3 fraction (> 10,000 fold) from DNA affinity chromatography and in the crude extracts prepared from γ and α IFN treated HeLa cells [Fu et al., *PROC. NATL. ACAD. SCI. USA*, **87** (1990)]. The antiserum was raised against a fusion protein [a cDNA fragment coding for part of 113 kD protein was inserted into pGEX-2T, a high expression vector in the E. coli [Smith et al., *PROC. NATL. ACAD. SCI. USA*, **83** (1986)] purified from E. coli [Smith et al., *GENE*, **67** (1988)]. The female NZW rabbits were immunized with 1 mg fusion protein in Freund's adjuvant. Two subsequent boosts two weeks apart were carried out with 500 mg fusion protein. The Western blot was carried out with conditions described previously [Pine et al., *MOL. CELL. BIOL.*, **10** (1990)].

FIGURE 10(B) presents the results of a mobility shift assay showing that the anti-113 antiserum affects the ISGF-3 shift complex. Preimmune serum or the 113 kD antiserum was added to shift reaction carried out as described [Fu et al. *PROC. NATL. ACAD. SCI. USA*, **87** (1990); Kessler et al. *GENES & DEV.*, **4**, (1990)] at room temperature for 20 min. then one-third of reaction material was loaded onto a 5% polyacrylamide gel. In addition unlabeled probe was included in one reaction to show specificity of the gel shift complexes.

FIGURE 11 shows the results of experiments investigating the IFN-α dependent phosphorylation of 113, 91 and 84 kD proteins. Protein samples from cells treated in various ways after 60 min. exposure to $^{32}PO_4^{-3}$ were precipitated with antiserum to 113 kD protein. Lane 1, no treatment of cells; Lane 2, cells treated 7 min. with IFN-α. By comparison with the marker proteins labeled 200, 97.5, 69 and 46 kD (kilo daltons), the PO_4^{-3} labeled proteins in the precipitate are seen to be 113 and 91 kD. Lane 3, cells treated with IFN-γ overnight (no phosphorylated proteins) and then (Lane 4) treated with IFN-α for 7 min. show heavier phosphorylation of 113, 91 and 84 kD.

FIGURE 12 is a chromatogram depicting the identification of phosphoamino acid. Phosphate labeled protein of 113, 91 or 84 kD size was hydrolyzed and chromatographed to reveal newly labeled phosphotyrosine. Cells untreated with IFN showed only phosphoserine label. (P Ser = phosphoserine; p Thr = phosphothreonine; P Tyr = phosphotyrosine.

FIGURE 13 depicts the characterization of GAF by gel mobility shift assays. **Panel A)** GAF specifically binds to GAS oligonucleotide in response to IFN-γ. Mobility shift assays of ^{32}P labeled GAS were performed with nuclear extracts from untreated FS2 fibroblasts (lane 1); treated with IFN-α for 15 min. (lane 2). Extracts from cells treated with IFN-γ for 15 min. were used for other gel mobility shift assays (lane 3-5). A fifty-fold excess of unlabeled GAS oligonucleotide (lane 4) of unlabeled oligonucleotide representing the ISRE (oligo 015, ref. 5; lane 5) were used for competition.

Panel B) Induction of GAF is independent of protein synthesis. Shift assays with labeled GAS and nuclear extracts from untreated cells (lane 1) cells treated with IFN-γ for 15 min. (lane 2), cells treated with IFN-γ for 15 min. in the presence of cycloheximide (lane 3).

Panel C) Time course of GAF activation. Nuclear extracts from cells treated with IFN-γ for the indicated times were used in gel mobility shift assays with ^{32}P labeled GAS.

Methods: The following double-stranded GAS oligonucleotide from the GBP promoter (15) was used in gel mobility shift assays:

5' CATGAGTTTCATATTACTCTAAATC 3'

3' TCAAAGTATAATGAGATTTAGGTAC 3'

The oligonucleotide was labelled with ^{32}P dCTP, dATP, dGTP, dTTP using the Klenow DNA polymerase. One ng of labeled oligonucleotide was mixed with 2 mg poly (didC) in 11.5 ml of gel mobility shift buffer containing 20 mM HEPES (pH 7.9), 4% Ficoll, 1 mM $MgCl_2$, 40mM KCl, 0.1 mM EGTA, 0.5 mM DTT. One ml of nuclear extract was added per sample and the binding reaction was carried out at room temperature for 20 min. Five ml of the reaction mixture was analyzed on 4% polyacrylamide gels. Nuclear extracts were prepared from FS2 human diploid fibroblasts (14). Human recombinant IFN-γ (gift of Dr. D. Vapnek of Amgen) was added to a final concentration of 5 ng/ml. IFN-α, (gift of P. Sorter, Hoffman La Roche) was used at 500 antiviral units per ml. Cycloheximide (50 mg/ml) was added before addition of IFN.

FIGURE 14 presents the identification of the 91 kD protein in the GAF shift complex.

Panel A) UV cross-link analysis of GAF. UV cross-linking analysis using N_3dUTP substituted oligonucleotide was described (25). Briefly oligonucleotide

5' AGTTTCATATTACTCTAAA 3'

3' TCAAAGTATAATGAGATTTAGGTAC 5'

was labeled with 5-N_3dUTP and ^{32}P dATP, dGTP, dCTP using the Klenow DNA polymerase. The N_3dUTP substituted oligonucleotide was mixed with nuclear extracts from IFN-γ treated cells (as in Fig. 13) for a mobility shift assay. Autoradiography was carried out while the gel was wet. The gel was then UV radiated for 5 min. in a Stratagene UV linker and the gel slice corresponding to the GAF complex was cut out and analysed on 7% SDS PAGE. Lane 1, ^{14}C-protein marker (Amersham); lane 2, GAF-DNA complex; lane 3, 100-fold excess of cold GAS oligonucleotide was included in shift reaction mixture; lane 4, no proteins were included in shift reaction mixture.

Panel B) GAF shift complex is specifically affected by antisera against the 91 kD protein. Mobility shift gel assays with nuclear extracts from cells treated with IFN-γ for 15 min. were carried out as described in Fig. 13 with various additions: Lane 1, no addition; lane 2, a fifty fold excess of unlabeled GAS oligonucleotide; lanes 3 and 5, preimmune sera; lane 4, antiserum against C-terminal 36 amino acid of 91 kD protein (91c); lane 6, antiserum against 91 kD protein (91m, amino acids 591-703). All sera were added at 1/120 final dilution.

Panel C) The 91 kD protein is present in the GAF gel shift complex. The protein in the GAF shift complex was analyzed by two-dimensional gel mobility shift-SDS electrophoresis followed by immuno-blotting. Partially purified GAF (see below) was used in a gel mobility shift assay (left panel, lane 1-3) using ^{32}P-labeled GAS oligonucleotide. In lane 2, no probe was added (A); lane 3, competition with 50-fold excess of cold GAS oligonucleotide to identify specificity of the GAF shift band. After electrophoresis and autoradiography, lanes 1 and 2 were cut out, rotated as indicated and directly subjected to 7% SDS-PAGE analysis (right upper panel and right lower panel, respectively). The gel was then electroblotted to nitrocellulose and detected with the antiserum against the 91 kD protein, using ECL kit (Amersham) to detect that protein. The position in the second gel that corresponded to the GAF shift complex in the first gel is indicated by an arrow.

Panel D) Analysis of ^{35}S labeled GAF. Fibroblasts (strain FS2) were labeled for 14 hours with ^{35}S methionine and treated with IFN-γ for 15 min. Nuclear extracts were prepared and ^{35}S-labeled proteins that would contain GAF were collected on biotinylated GAS oligonucleutide bound to beads. After elution, the affinity purified sample was used to analyze ^{35}S proteins by the two-dimensional gel mobility shift-SDS PAGE analysis described in Fig. 14C. The left panel shows gel mobility shift using ^{35}S labeled affinity purified sample (lane 1-3). Preimmune (lane 2) or immuserum (lane 3) against the 91 kD protein was added. Lanes 2 and 3 were cut out and further analyzed by SDS PAGE followed by autoradiography to expose ^{35}S labeled proteins. The position of the 91 kD protein is indicated by a dashed arrow.

Methods: Purification of GAF using biotinylated GAS oligonucleotde was carried out essentially as described (18). 5'-biotinylated double-stranded GAS oligonucleotide (American Synthesis) was mixed with crude extracts in gel mobility shift buffer (Fig. 13) and incubated at room temperature for 20 min. The reaction mixture was then incubated with streptoavidin-agarose beads (Sigma) and rotated at 40°C for 2 hrs. The beads were collected and washed four times with gel mobility shift buffer. The proteins bound to the beads were eluted first with E(0.2) buffer containing 50 mM Tris (pH 7.6), 1 mM DTT, 10% glycerol, 0.2 M NaCl, 0.5 mM EDTA and then eluted with E(0.8) buffer which is same as E (0-2) except it contained 0.8 NaCl. The fraction eluted from E(O.8) contains GAF activity and was used for further analysis.

Human fibroblasts were grown in Dulbecco's modified Eagle's medium (Gibco) containing 10% bovine serum. Cells were labelled with 0.1 mCi/ml ^{35}S-labeled methionine in medium lacking L-methionine and cysteine. Labeling medium was removed by washing twice with ice-cold phosphate-buffered saline (PBS). Nuclear extracts were prepared as in Fig. 13.

FIGURE 15 shows that IFN-γ causes nuclear localization of the 91 kD protein but not the 113 kD protein in human fibroblast FS2 cells. Untreated (A and C) and IFN-γ treated cells (B and D) were stained with either an anti-91 kD protein antibody (91c, A and B) or an anti-113 kD protein antibody (C and D). Cells were cultured as described (Fig. 14) in 8 well tissue culture chamber slides. Twenty min. before fixation cells were treated with IFN-γ (5 ng/ml), rinsed 2x in PBS and fixed in a solution of methanol acetone for 2 min. After 2 washes in TBST (10 mM Tris-Cl pH 8.0, 100 mM NaCl, 0.02% Tween 20) cells were blocked for 40 min. in TBST+3% BSA. Primary antibody was added (anti 113 or anti 91; 1/100 final dilution) in blocking buffer for 2.5 hrs. After 3 washes in TBST, secondary antibody (donkey anti-rabbit fluorescein conjugated antibody) was added (1/200 dilution final conc.) for 70 min. at room temperature. After 3

washes in TBST, cells were rinsed in 0.1 x PBS, 90% glycerol, 0.1% P-phenylenediamine pH 8.0 and dried.

FIGURE 16 shows the activation of GAF by phosphorylation.

Panel A) Time course of IFN-γ induction analyzed by immunoblotting. Nuclear extracts from cells treated with IFN-γ at indicated times were prepared. Five mg of protein from each sample was analyzed for 91 kD protein by Western blot (7% SDS-PAGE, electroblotted to nitrocellulose, detection of 91 kD protein with specific antiserum to 91 kD protein by ECL, Amersham). The slower and faster migratng forms are indicated.

Panel B) Inhibitor and enzymatic evidence for phosphorylation of 91 kD protein. Lanes 1-3: Immunoblot on crude cell extracts as in panel A of cells treated as indicated; staurosporine treatment was at 0.5 mM and IFN-γ induction was 15 min. Lanes 4-6: Immunoblot on partially purified GAF (as in Fig. 14, lane 6) or GAF that was treated (lane 5) with calf intestinal phosphatase (CIP; 1.8 units/ml; 30', 30°); Lane 4 was an incubation control lacking CIP.

Panel C)

Effect of staurosporine and phosphatase treatment on the GAF DNA binding analyzed by gel mobility shift assays. As in panel B samples were analyzed by gel mobility shift assays as described in FIGURE 13.

FIGURE 17 shows the IFN-γ dependent tyrosine phosphorylation of the 91 kD protein.

Panel A) Immunoprecipitation of ^{35}S and ^{32}P labeled 91 kD protein. Cells were labeled with ^{35}S methionine for 4 hours as described in Fig. 14 or with ^{32}P for 1.5 hr. [0.5 mci/ml ^{32}P orthophosphate (Amersham) in medium otherwise lacking phosphate]. Labeling medium was removed and cells washed twice with PBS and extracted in lysis buffer (50 mM Tris, pH 8.0, 280 mM NaCl, 0.05% NP-40, 0.2 mM EDTA, 2 mM EGTA, 10% glycerol, 1 mM DTT, 0.5 mM PMSF, 0.5 mg/ml leupeptin, 3 mg/ml aproteinin, 1 mg/ml pepstatin, 0.1 mM N_3VO_4). The extract was cleared with preimmune serum and protein A-G agarose (Oncogene

Science). The 91 kD protein was then immunoprecipitated with 91 kD antiserum. ^{35}S labeled (lane 1-3) and ^{32}P labeled (lanes 4-7) immunoprecipitates were then analyzed by 7% SDS-PAGE followed by autoradiography. Lane 1, untreated; lane 2, IFN-γ treated for 15 min.; lane 3, staurosporine pretreated for 10 min. followed by 15 min. IFN-γ treatment; lane 4, IFN-γ treated for 7 min.; lane 5, untreated; lane 6, IFN-γ treated for 15 min; lane 7, staurosporine pre-treated for 10 min. followed by 15 min. IFN-γ treatment. The slower migration and fast migration forms are indicated.

Panel B) Phosphoamino acid analysis of the 91 kD protein. The ^{32}P labeled 91 kD protein was cut out from SDS-PAGE gel (see Fig. 17A, lane 4 and 5). The ^{32}P-labeled protein as digested with 6N HCl for 1.5 hours at 110°C. Phosphoamino acids were analyzed as described (26). The migration of phosphoserine (p-ser), phosphothreonine (p-thr) and phosphotyrosine (p-tyr) is indicated.

FIGURE 18 presents the phosphopeptide mapping of thermolysin digests of the 91 kD protein. Peptide mapping was performed as described (26). Briefly, the ^{32}P labeled 91 kD protein was immunoprecipitated, separated by SDS-PAGE gel, eluted and digested with thermolysin (100 mg/ml).

DETAILED DESCRIPTION

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Maniatis, Fritsch & Sambrook, "Molecular Cloning: A Laboratory Manual" (1982); "DNA Cloning: A Practical Approach," Volumes I and II (D.N. Glover ed. 1985); "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription And Translation" [B.D. Hames & S.J. Higgins, eds. (1984)]; "Animal Cell Culture"

[R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984).

Therefore, if appearing herein, the following terms shall have the definitions set out below.

The terms "receptor recognition factor", "receptor recognition-tyrosine kinase factor", "receptor recognition factor/tyrosine kinase substrate", "receptor recognition/transcription factor", "recognition factor" and "recognition factor protein(s)" and any variants not specifically listed, may be used herein interchangeably, and as used throughout the present application and claims refer to proteinaceous material including single or multiple proteins, and extends to those proteins having the DNA and amino acid sequence data described herein and presented in FIGURE 1 (SEQ ID NO:1), FIGURE 2 (SEQ ID NO:2) and in FIGURE 3 (SEQ ID NO:3), and the profile of activities set forth herein and in the Claims. Accordingly, proteins displaying substantially equivalent or altered activity are likewise contemplated. These modifications may be deliberate, for example, such as modifications obtained through site-directed mutagenesis, or may be accidental, such as those obtained through mutations in hosts that are producers of the complex or its named subunits. Also, the terms "receptor recognition factor", "recognition factor" and "recognition factor protein(s)" are intended to include within their scope proteins specifically recited herein as well as all substantially homologous analogs and allelic variations.

The amino acid residues described herein are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired fuctional property of immunoglobulin-binding is retained by the polypeptide. NH2 refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In keeping with standard

polypeptide nomenclature, *J. Biol. Chem.*, **243**:3552-59 (1969), abbreviations for amino acid residues are shown in the following Table of Correspondence:

TABLE OF CORRESPONDENCE

SYMBOL		AMINO ACID
1-Letter	3-Letter	
Y	Tyr	tyrosine
G	Gly	glycine
F	Phe	phenylalanine
M	Met	methionine
A	Ala	alanine
S	Ser	serine
I	Ile	isoleucine
L	Leu	leucine
T	Thr	threonine
V	Val	valine
P	Pro	proline
K	Lys	lysine
H	His	histidine
Q	Gln	glutamine
E	Glu	glutamic acid
W	Trp	tryptophan
R	Arg	arginine
D	Asp	aspartic acid
N	Asn	asparagine
C	Cys	cysteine

It should be noted that all amino-acid residue sequences are represented herein by formulae whose left and right orientation is in the conventional direction of amino-terminus to carboxy-terminus. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a

further sequence of one or more amino-acid residues. The above Table is presented to correlate the three-letter and one-letter notations which may appear alternately herein.

A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*; i.e., capable of replication under its own control.

A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form, or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA).

An "origin of replication" refers to those DNA sequences that participate in DNA synthesis.

A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not

limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

An "expression control sequence" is a DNA sequence that controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence.

A "signal sequence" can be included before the coding sequence. This sequence encodes a signal peptide, N-terminal to the polypeptide, that communicates to the host cell to direct the polypeptide to the cell surface or secrete the polypeptide into

the media, and this signal peptide is clipped off by the host cell before the protein leaves the cell. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

The term "oligonucleotide", as used herein in referring to the probe of the present invention, is defined as a molecule comprised of two or more ribonucleotides, preferably more than three. Its exact size will depend upon many factors which, in turn, depend upon the ultimate function and use of the oligonucleotide.

The term "primer" as used herein refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product, which is complementary to a nucleic acid strand, is induced, i.e., in the presence of nucleotides and an inducing agent such as a DNA polymerase and at a suitable temperature and pH. The primer may be either single-stranded or double-stranded and must be sufficiently long to prime the synthesis of the desired extension product in the presence of the inducing agent. The exact length of the primer will depend upon many factors, including temperature, source of primer and use of the method. For example, for diagnostic applications, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides.

The primers herein are selected to be "substantially" complementary to different strands of a particular target DNA sequence. This means that the primers must be sufficiently complementary to hybridize with their respective strands. Therefore, the primer sequence need not reflect the exact sequence of the template. For example, a non-complementary nucleotide fragment may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer sequence has sufficient

complementarity with the sequence of the strand to hybridize therewith and thereby form the template for the synthesis of the extension product.

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to bacterial enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence.

A cell has been "transformed" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. The transforming DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

Two DNA sequences are "substantially homologous" when at least about 75% (preferably at least about 80%, and most preferably at least about 90 or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Maniatis et al., *supra*; DNA Cloning, Vols. I & II, *supra*; Nucleic Acid Hybridization, *supra*.

A "heterologous" region of the DNA construct is an identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. Another example of a heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., a cDNA where the genomic coding sequence contains introns, or synthetic sequences having codons different than the native gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

An "antibody" is any immunoglobulin, including antibodies and fragments thereof, that binds a specific epitope. The term encompasses polyclonal, monoclonal, and chimeric antibodies, the last mentioned described in further detail in U.S. Patent Nos. 4,816,397 and 4,816,567.

An "antibody combining site" is that structural portion of an antibody molecule comprised of heavy and light chain variable and hypervariable regions that specifically binds antigen.

The phrase "antibody molecule" in its various grammatical forms as used herein contemplates both an intact immunoglobulin molecule and an immunologically active portion of an immunoglobulin molecule.

Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contains the paratope, including those portions known in the art as Fab, Fab', $F(ab')_2$ and F(v), which portions are preferred for use in the therapeutic methods described herein.

Fab and $F(ab')_2$ portions of antibody molecules are prepared by the proteolytic reaction of papain and pepsin, respectively, on substantially intact antibody molecules by methods that are well-known. See for example, U.S. Patent No. 4,342,566 to Theofilopolous et al. Fab' antibody molecule portions are also well-known and are produced from $F(ab')_2$ portions followed by reduction of the disulfide bonds linking the two heavy chain portions as with mercaptoethanol, and followed by alkylation of the resulting protein mercaptan with a reagent such as iodoacetamide. An antibody containing intact antibody molecules is preferred herein.

The phrase "monoclonal antibody" in its various grammatical forms refers to an antibody having only one species of antibody combining site capable of immunoreacting with a particular antigen. A monoclonal antibody thus typically displays a single binding affinity for any antigen with which it immunoreacts. A monoclonal antibody may therefore contain an antibody molecule having a plurality of antibody combining sites, each immunospecific for a different antigen; e.g., a bispecific (chimeric) monoclonal antibody.

The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human.

The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to prevent, and preferably reduce by at least about 30 percent, more preferably by at least 50 percent, most preferably by at least 90 percent, a clinically significant change in the S phase activity of a target cellular mass, or other feature of pathology such as for example, elevated blood pressure, fever or white cell count as may attend its presence and activity.

A DNA sequence is "operatively linked" to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to be expressed and maintaining the correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start signal, such a start signal can be inserted in front of the gene.

The term "standard hybridization conditions" refers to salt and temperature conditions substantially equivalent to 5 x SSC and 65°C for both hybridization and wash.

In its primary aspect, the present invention concerns the identification of a receptor recognition factor, and the isolation and sequencing of particular receptor recognition factor proteins, that is believed to be present in cytoplasm and that serves as a signal transducer between a particular cellular receptor having bound thereto an equally specific polypeptide ligand, and the comparably specific transcription factor that enters the nucleus of the cell and interacts with a specific DNA binding site for the activation of the gene to promote the predetermined response to the particular polypeptide stimulus. The present disclosure confirms that specific and individual receptor recognition factors exist that correspond to known stimuli such as tumor necrosis factor, nerve growth factor, platelet-derived growth factor and the like. Specific evidence of this is set forth herein with respect to the interferons α and γ (IFNα and IFNγ).

The present receptor recognition factor is likewise noteworthy in that it appears not to be demonstrably affected by fluctuations in second messenger activity and concentration. The receptor recognition factor proteins appear to act as a substrate

for tyrosine kinase domains, however do not appear to interact with G-proteins, and therefore do not appear to be second messengers.

A particular receptor recognition factor identified herein by SEQ ID NO:2, has been determined to be present in cytoplasm and serves as a signal transducer and a specifice transcription factor in response to IFN-γ stimulation that enters the nucleus of the cell and interacts directly with a specific DNA binding site for the activation of the gene to promote the predetermined response to the particular polypeptide stimulus. This particular factor also acts as a translation protein and, in particular, as a DNA binding protein in response to interferon-γ stimulation. This factor is likewise noteworthy in that it has the following characteristics:

a) It interacts with an interferon-γ-bound receptor kinase complex;

b) It is a tyrosine kinase substrate; and

c) When phosphorylated, it serves as a DNA binding protein.

More particularly, the factor of SEQ ID NO:2 directly interacts with DNA after acquiring phosphate on tyrosine located at or about position 690 of the amino acid sequence. Also, interferon-γ-dependent activation of this factor occurs without new protein synthesis and appears within minutes of interferon-γ treatment, achieves maximum extent between 15 and 30 minutes thereafter, and then disappears after 2-3 hours.

As stated above, the present invention relates to a recombinant DNA molecule or cloned gene, or a degenerate variant thereof, which encodes a receptor recognition factor, or a fragment thereof, that possesses a molecular weight and DNA sequence selected from a molecular weight of about 113 kD and the DNA sequence set forth in FIGURE 1 (SEQ ID NO:1), a molecular weight of about 91 kD and the DNA sequence set forth in FIGURE 2 (SEQ ID NO:2), and a molecular weight of about 84 kD and the DNA sequence set forth in FIGURE 3 (SEQ ID NO:3).

The possibilities both diagnostic and therapeutic that are raised by the existence of the receptor recognition factor or factors, derive from the fact that the factors appear to participate in direct and causal protein-protein interaction between the receptor that is occupied by its ligand, and those factors that thereafter directly interface with the gene and effect transcription and accordingly gene activation. As suggested earlier and elaborated further on herein, the present invention contemplates pharmaceutical intervention in the cascade of reactions in which the receptor recognition factor is implicated, to modulate the activity initiated by the stimulus bound to the cellular receptor.

Thus, in instances where it is desired to reduce or inhibit the gene activity resulting from a particular stimulus or factor, an appropriate inhibitor of the receptor recognition factor could be introduced to block the interaction of the receptor recognition factor with those factors causally connected with gene activation. Correspondingly, instances where insufficient gene activation is taking place could be remedied by the introduction of additional quantities of the receptor recognition factor or its chemical or pharmaceutical cognates, analogs, fragments and the like.

As discussed earlier, the recognition factors or thir binding partners or other ligands or agents exhibiting either mimicry or antagonism to the recognition factors or control over their production, may be prepared in pharmaceutical compositions, with a suitable carrier and at a strength effective for administration by various means to a patient experiencing an adverse medical condition associated specific transcriptional stimulation for the treatment thereof. A variety of administrative techniques may be utilized, among them parenteral techniques such as subcutaneous, intravenous and intraperitoneal injections, catheterizations and the like. Average quantities of the recognition factors or their subunits may vary and in particular should be based upon the recommendations and prescription of a qualified physician or veterinarian.

Also, antibodies including both polyclonal and monoclonal antibodies, and drugs that modulate the production or activity of the recognition factors and/or their subunits may possess certain diagnostic applications and may for example, be utilized for the purpose of detecting and/or measuring conditions such as viral infection or the like. For example, the recognition factor or its subunits may be used to produce both polyclonal and monoclonal antibodies to themselves in a variety of cellular media, by known techniques such as the hybridoma technique utilizing, for example, fused mouse spleen lymphocytes and myeloma cells. Likewise, small molecules that mimic or antagonize the activity(ies) of the receptor recognition factors of the invention may be discovered or synthesized, and may be used in diagnostic and/or therapeutic protocols.

The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal, antibody-producing cell lines can also be created by techniques other than fusion, such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g., M. Schreier et al., "Hybridoma Techniques" (1980); Hammerling et al., "Monoclonal Antibodies And T-cell Hybridomas" (1981); Kennett et al., "Monoclonal Antibodies" (1980); see also U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,451,570; 4,466,917; 4,472,500; 4,491,632; 4,493,890.

Panels of monoclonal antibodies produced against recognition factor peptides can be screened for various properties; i.e., isotype, epitope, affinity, etc. Of particular interest are monoclonal antibodies that neutralize the activity of the recognition factor or its subunits. Such monoclonals can be readily identified in recognition factor activity assays. High affinity antibodies are also useful when immunoaffinity purification of native or recombinant recognition factor is possible.

Preferably, the anti-recognition factor antibody used in the diagnostic methods of this invention is an affinity purified polyclonal antibody. More preferably, the antibody is a monoclonal antibody (mAb). In addition, it is preferable for the

anti- recognition factor antibody molecules used herein be in the form of Fab, Fab', $F(ab')_2$ or F(v) portions of whole antibody molecules.

As suggested earlier, the diagnostic method of the present invention comprises examining a cellular sample or medium by means of an assay including an effective amount of an antagonist to a receptor recognition factor/protein, such as an anti-recognition factor antibody, preferably an affinity-purified polyclonal antibody, and more preferably a mAb. In addition, it is preferable for the anti-recognition factor antibody molecules used herein be in the form of Fab, Fab', $F(ab')_2$ or F(v) portions or whole antibody molecules. As previously discussed, patients capable of benefiting from this method include those suffering from cancer, a pre-cancerous lesion, a viral infection or other like pathological derangement. Methods for isolating the recognition factor and inducing anti-recognition factor antibodies and for determining and optimizing the ability of anti-recognition factor antibodies to assist in the examination of the target cells are all well-known in the art.

Methods for producing polyclonal anti-polypeptide antibodies are well-known in the art. See U.S. Patent No. 4,493,795 to Nestor et al. A monoclonal antibody, typically containing Fab and/or $F(ab')_2$ portions of useful antibody molecules, can be prepared using the hybridoma technology described in *Antibodies - A Laboratory Manual*, Harlow and Lane, eds., Cold Spring Harbor Laboratory, New York (1988), which is incorporated herein by reference. Briefly, to form the hybridoma from which the monoclonal antibody composition is produced, a myeloma or other self-perpetuating cell line is fused with lymphocytes obtained from the spleen of a mammal hyperimmunized with a recognition factor-binding portion thereof, or recognition factor, or an origin-specific DNA-binding portion thereof.

Splenocytes are typically fused with myeloma cells using polyethylene glycol (PEG) 6000. Fused hybrids are selected by their sensitivity to HAT. Hybridomas

producing a monoclonal antibody useful in practicing this invention are identified by their ability to immunoreact with the present recognition factor and their ability to inhibit specified transcriptional activity in target cells.

A monoclonal antibody useful in practicing the present invention can be produced by initiating a monoclonal hybridoma culture comprising a nutrient medium containing a hybridoma that secretes antibody molecules of the appropriate antigen specificity. The culture is maintained under conditions and for a time period sufficient for the hybridoma to secrete the antibody molecules into the medium. The antibody-containing medium is then collected. The antibody molecules can then be further isolated by well-known techniques.

Media useful for the preparation of these compositions are both well-known in the art and commercially available and include synthetic culture media, inbred mice and the like. An exemplary synthetic medium is Dulbecco's minimal essential medium (DMEM; Dulbecco et al., *Virol.* **8**:396 (1959)) supplemented with 4.5 gm/l glucose, 20 mm glutamine, and 20% fetal calf serum. An exemplary inbred mouse strain is the Balb/c.

Methods for producing monoclonal anti-recognition factor antibodies are also well-known in the art. See Niman et al., *Proc. Natl. Acad. Sci. USA*, **80**:4949-4953 (1983). Typically, the present recognition factor or a peptide analog is used either alone or conjugated to an immunogenic carrier, as the immunogen in the before described procedure for producing anti-recognition factor monoclonal antibodies. The hybridomas are screened for the ability to produce an antibody that immunoreacts with the recognition factor peptide analog and the present recognition factor.

The present invention further contemplates therapeutic compositions useful in practicing the therapeutic methods of this invention. A subject therapeutic composition includes, in admixture, a pharmaceutically acceptable excipient

(carrier) and one or more of a receptor recognition factor, polypeptide analog thereof or fragment thereof, as described herein as an active ingredient. In a preferred embodiment, the composition comprises an antigen capable of modulating the specific binding of the present recognition factor within a target cell.

The preparation of therapeutic compositions which contain polypeptides, analogs or active fragments as active ingredients is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active ingredient.

A polypeptide, analog or active fragment can be formulated into the therapeutic composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide or antibody molecule) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The therapeutic polypeptide-, analog- or active fragment-containing compositions are conventionally administered intravenously, as by injection of a unit dose, for

example. The term "unit dose" when used in reference to a therapeutic composition of the present invention refers to physically discrete units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e., carrier, or vehicle.

The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to utilize the active ingredient, and degree of inhibition or neutralization of recognition factor binding capacity desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosages may range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably one to several, milligrams of active ingredient per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations of ten nanomolar to ten micromolar in the blood are contemplated.

The therapeutic compositions may further include an effective amount of the factor/factor synthesis promoter antagonist or analog thereof, and one or more of the following active ingredients: an antibiotic, a steroid. Exemplary formulations are given below:

Formulations

Intravenous Formulation I Ingredient	mg/ml
cefotaxime	250.0
receptor recognition factor	10.0
dextrose USP	45.0
sodium bisulfite USP	3.2
edetate disodium USP	0.1
water for injection q.s.a.d.	1.0ml

Intravenous Formulation II Ingredient	mg/ml
ampicillin	250.0
receptor recognition factor	10.0
sodium bisulfite USP	3.2
disodium edetate USP	0.1
water for injection q.s.a.d.	1.0ml

Intravenous Formulation III Ingredient	mg/ml
gentamicin (charged as sulfate)	40.0
receptor recognition factor	10.0
sodium bisulfite USP	3.2
disodium edetate USP	0.1
water for injection q.s.a.d.	1.0ml

Intravenous Formulation IV Ingredient	mg/ml
recognition factor	10.0
dextrose USP	45.0

sodium bisulfite USP	3.2
edetate disodium USP	0.1
water for injection q.s.a.d.	1.0 ml

Intravenous Formulation V

Ingredient	mg/ml
recognition factor antagonist	5.0
sodium bisulfite USP	3.2
disodium edetate USP	0.1
water for injection q.s.a.d.	1.0 ml

As used herein, "pg" means picogram, "ng" means nanogram, "ug" or "μg" mean microgram, "mg" means milligram, "ul" or "μl" mean microliter, "ml" means milliliter, "l" means liter.

Another feature of this invention is the expression of the DNA sequences disclosed herein. As is well known in the art, DNA sequences may be expressed by operatively linking them to an expression control sequence in an appropriate expression vector and employing that expression vector to transform an appropriate unicellular host.

Such operative linking of a DNA sequence of this invention to an expression control sequence, of course, includes, if not already part of the DNA sequence, the provision of an initiation codon, ATG, in the correct reading frame upstream of the DNA sequence.

A wide variety of host/expression vector combinations may be employed in expressing the DNA sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and Synthetic DNA sequences. Suitable vectors include derivatives of SV40 and known bacterial plasmids, e.g., *E. coli* plasmids col El, pCR1, pBR322, pMB9

and their derivatives, plasmids such as RP4; phage DNAS, e.g., the numerous derivatives of phage λ, e.g., NM989, and other phage DNA, e.g., M13 and Filamentous single stranded phage DNA; yeast plasmids such as the 2μ plasmid or derivatives thereof; vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAS, such as plasmids that have been modified to employ phage DNA or other expression control sequences; and the like.

Any of a wide variety of expression control sequences -- sequences that control the expression of a DNA sequence operatively linked to it -- may be used in these vectors to express the DNA sequences of this invention. Such useful expression control sequences include, for example, the early or late promoters of SV40, CMV, vaccinia, polyoma or adenovirus, the *lac* system, the *trp* system, the *TAC* system, the *TRC* system, the *LTR* system, the major operator and promoter regions of phage λ, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase (e.g., Pho5), the promoters of the yeast α-mating factors, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof.

A wide variety of unicellular host cells are also useful in expressing the DNA sequences of this invention. These hosts may include well known eukaryotic and prokaryotic hosts, such as strains of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, fungi such as yeasts, and animal cells, such as CHO, Rl.l, B-W and L-M cells, African Green Monkey kidney cells (e.g., COS 1, COS 7, BSC1, BSC40, and BMT10), insect cells (e.g., Sf9), and human cells and plant cells in tissue culture.

It will be understood that not all vectors, expression control sequences and hosts will function equally well to express the DNA sequences of this invention. Neither will all hosts function equally well with the same expression system.

However, one skilled in the art will be able to select the proper vectors, expression control sequences, and hosts without undue experimentation to accomplish the desired expression without departing from the scope of this invention. For example, in selecting a vector, the host must be considered because the vector must function in it. The vector's copy number, the ability to control that copy number, and the expression of any other proteins encoded by the vector, such as antibiotic markers, will also be considered.

In selecting an expression control sequence, a variety of factors will normally be considered. These include, for example, the relative strength of the system, its controllability, and its compatibility with the particular DNA sequence or gene to be expressed, particularly as regards potential secondary structures. Suitable unicellular hosts will be selected by consideration of, e.g., their compatibility with the chosen vector, their secretion characteristics, their ability to fold proteins correctly, and their fermentation requirements, as well as the toxicity to the host of the product encoded by the DNA sequences to be expressed, and the ease of purification of the expression products.

Considering these and other factors a person skilled in the art will be able to construct a variety of vector/expression control sequence/host combinations that will express the DNA sequences of this invention on fermentation or in large scale animal culture.

It is further intended that receptor recognition factor analogs may be prepared from nucleotide sequences of the protein complex/subunit derived within the scope of the present invention. Analogs, such as fragments, may be produced, for example, by pepsin digestion of receptor recognition factor material. Other analogs, such as muteins, can be produced by standard site-directed mutagenesis of receptor recognition factor coding sequences. Analogs exhibiting "receptor recognition factor activity" such as small molecules, whether functioning as promoters or inhibitors, may be identified by known *in vivo* and/or *in vitro* assays.

As mentioned above, a DNA sequence encoding receptor recognition factor can be prepared synthetically rather than cloned. The DNA sequence can be designed with the appropriate codons for the receptor recognition factor amino acid sequence. In general, one will select preferred codons for the intended host if the sequence will be used for expression. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge, *Nature*, **292**:756 (1981); Nambair et al., *Science*, **223**:1299 (1984); Jay et al., *J. Biol. Chem.*, *259*:6311 (1984).

Synthetic DNA sequences allow convenient construction of genes which will express receptor recognition factor analogs or "muteins". Alternatively, DNA encoding muteins can be made by site-directed mutagenesis of native receptor recognition factor genes or cDNAs, and muteins can be made directly using conventional polypeptide synthesis.

A general method for site-specific incorporation of unnatural amino acids into proteins is described in Christopher J. Noren, Spencer J. Anthony-Cahill, Michael C. Griffith, Peter G. Schultz, *Science*, **244**:182-188 (April 1989). This method may be used to create analogs with unnatural amino acids.

The present invention extends to the preparation of antisense nucleotides and ribozymes that may be used to interfere with the expression of the receptor recognition proteins at the translational level. This approach utilizes antisense nucleic acid and ribozymes to block translation of a specific mRNA, either by masking that mRNA with an antisense nucleic acid or cleaving it with a ribozyme.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule. (See Weintraub, 1990; Marcus-Sekura, 1988.) In the cell, they hybridize to that mRNA, forming a double stranded molecule. The cell does not translate an mRNA in this double-stranded form. Therefore, antisense nucleic acids interfere with the expression of mRNA

into protein. Oligomers of about fifteen nucleotides and molecules that hybridize to the AUG initiation codon will be particularly efficient, since they are easy to synthesize and are likely to pose fewer problems than larger molecules when introducing them into receptor recognition factor-producing cells. Antisense methods have been used to inhibit the expression of many genes *in vitro* (Marcus-Sekura, 1988; Hambor et al., 1988).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single stranded RNA molecules in a manner somewhat analogous to DNA restriction endonucleases. Ribozymes were discovered from the observation that certain mRNAs have the ability to excise their own introns. By modifying the nucleotide sequence of these RNAs, researchers have been able to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, 1988.). Because they are sequence-specific, only mRNAs with particular sequences are inactivated.

Investigators have identified two types of ribozymes, *Tetrahymena*-type and "hammerhead"-type. (Hasselhoff and Gerlach, 1988) *Tetrahymena*-type ribozymes recognize four-base sequences, while "hammerhead"-type recognize eleven- to eighteen-base sequences. The longer the recognition sequence, the more likely it is to occur exclusively in the target mRNA species. Therefore, hammerhead-type ribozymes are preferable to *Tetrahymena*-type ribozymes for inactivating a specific mRNA species, and eighteen base recognition sequences are preferable to shorter recognition sequences.

The DNA sequences described herein may thus be used to prepare antisense molecules against, and ribozymes that cleave mRNAs for receptor recognition factor proteins and their ligands.

The present invention also relates to a variety of diagnostic applications, including methods for detecting the presence of stimuli such as the earlier referenced

polypeptide ligands, by reference to their ability to elicit the activities which are mediated by the present receptor recognition factor. As mentioned earlier, the receptor recognition factor can be used to produce antibodies to itself by a variety of known techniques, and such antibodies could then be isolated and utilized as in tests for the presence of particular transcriptional activity in suspect target cells.

As described in detail above, antibody(ies) to the receptor recognition factor can be produced and isolated by standard methods including the well known hybridoma techniques. For convenience, the antibody(ies) to the receptor recognition factor will be referred to herein as Ab_1 and antibody(ies) raised in another species as Ab_2.

The presence of receptor recognition factor in cells can be ascertained by the usual immunological procedures applicable to such determinations. A number of useful procedures are known. Three such procedures which are especially useful utilize either the receptor recognition factor labeled with a detectable label, antibody Ab_1 labeled with a detectable label, or antibody Ab_2 labeled with a detectable label. The procedures may be summarized by the following equations wherein the asterisk indicates that the particle is labeled, and "RRF" stands for the receptor recognition factor:

A. $RRF^* + Ab_1 = RRF^*Ab_1$

B. $RRF + Ab^* = RRFAb_1^*$

C. $RRF + Ab_1 + Ab_2^* = RRFAb_1Ab_2^*$

The procedures and their application are all familiar to those skilled in the art and accordingly may be utilized within the scope of the present invention. The "competitive" procedure, Procedure A, is described in U.S. Patent Nos. 3,654,090 and 3,850,752. Procedure C, the "sandwich" procedure, is described in U.S. Patent Nos. RE 31,006 and 4,016,043. Still other procedures are known such as the "double antibody", or "DASP" procedure.

In each instance, the receptor recognition factor forms complexes with one or more antibody(ies) or binding partners and one member of the complex is labeled with a detectable label. The fact that a complex has formed and, if desired, the amount thereof, can be determined by known methods applicable to the detection of labels.

It will be seen from the above, that a characteristic property of Ab_2 is that it will react with Ab_1. This is because Ab_1 raised in one mammalian species has been used in another species as an antigen to raise the antibody Ab_2. For example, Ab_2 may be raised in goats using rabbit antibodies as antigens. Ab_2 therefore would be anti-rabbit antibody raised in goats. For purposes of this description and claims, Ab_1 will be referred to as a primary or anti-receptor recognition factor antibody, and Ab_2 will be referred to as a secondary or anti-Ab_1 antibody.

The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals which fluoresce when exposed to ultraviolet light, and others.

A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine and auramine. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate.

The receptor recognition factor or its binding partner(s) can also be labeled with a radioactive element or with an enzyme. The radioactive label can be detected by any of the currently available counting procedures. The preferred isotope may be selected from ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re.

Enzyme labels are likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques. The enzyme is conjugated to the selected particle by

reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Many enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase, ß-glucuronidase, ß-D-glucosidase, ß-D-galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Patent Nos. 3,654,090; 3,850,752; and 4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

A particular assay system developed and utilized in accordance with the present invention, is known as a receptor assay. In a receptor assay, the material to be assayed is appropriately labeled and then certain cellular test colonies are inoculated with a quantity of both the labeled and unlabeled material after which binding studies are conducted to determine the extent to which the labeled material binds to the cell receptors. In this way, differences in affinity between materials can be ascertained.

Accordingly, a purified quantity of the receptor recognition factor may be radiolabeled and combined, for example, with antibodies or other inhibitors thereto, after which binding studies would be carried out. Solutions would then be prepared that contain various quantities of labeled and unlabeled uncombined receptor recognition factor, and cell samples would then be inoculated and thereafter incubated. The resulting cell monolayers are then washed, solubilized and then counted in a gamma counter for a length of time sufficient to yield a standard error of $<5\%$. These data are then subjected to Scatchard analysis after which observations and conclusions regarding material activity can be drawn. While the foregoing is exemplary, it illustrates the manner in which a receptor assay may be performed and utilized, in the instance where the cellular binding ability of the assayed material may serve as a distinguishing characteristic.

An assay useful and contemplated in accordance with the present invention is known as a "cis/trans" assay. Briefly, this assay employs two genetic constructs, one of which is typically a plasmid that continually expresses a particular receptor of interest when transfected into an appropriate cell line, and the second of which is a plasmid that expresses a reporter such as luciferase, under the control of a receptor/ligand complex. Thus, for example, if it is desired to evaluate a compound as a ligand for a particular receptor, one of the plasmids would be a construct that results in expression of the receptor in the chosen cell line, while the second plasmid would possess a promoter linked to the luciferase gene in which the response element to the particular receptor is inserted. If the compound under test is an agonist for the receptor, the ligand will complex with the receptor, and the resulting complex will bind the response element and initiate transcription of the luciferase gene. The resulting chemiluminescence is then measured photometrically, and dose response curves are obtained and compared to those of known ligands. The foregoing protocol is described in detail in U.S. Patent No. 4,981,784 and PCT International Publication No. WO 88/03168, for which purpose the artisan is referred.

In a further embodiment of this invention, commercial test kits suitable for use by a medical specialist may be prepared to determine the presence or absence of predetermined transcriptional activity or predetermined transcriptional activity capability in suspected target cells. In accordance with the testing techniques discussed above, one class of such kits will contain at least the labeled receptor recognition factor or its binding partner, for instance an antibody specific thereto, and directions, of course, depending upon the method selected, e.g., "competitive", "sandwich", "DASP" and the like. The kits may also contain peripheral reagents such as buffers, stabilizers, etc.

Accordingly, a test kit may be prepared for the demonstration of the presence or capability of cells for predetermined transcriptional activity, comprising:

(a) a predetermined amount of at least one labeled immunochemically reactive component obtained by the direct or indirect attachment of the present receptor recognition factor or a specific binding partner thereto, to a detectable label;

(b) other reagents; and

(c) directions for use of said kit.

More specifically, the diagnostic test kit may comprise:

(a) a known amount of the receptor recognition factor as described above (or a binding partner) generally bound to a solid phase to form an immunosorbent, or in the alternative, bound to a suitable tag, or plural such end products, etc. (or their binding partners) one of each;

(b) if necessary, other reagents; and

(c) directions for use of said test kit.

In a further variation, the test kit may be prepared and used for the purposes stated above, which operates according to a predetermined protocol (e.g. "competitive", "sandwich", "double antibody", etc.), and comprises:

(a) a labeled component which has been obtained by coupling the receptor recognition factor to a detectable label;

(b) one or more additional immunochemical reagents of which at least one reagent is a ligand or an immobilized ligand, which ligand is selected from the group consisting of:

(i) a ligand capable of binding with the labeled component (a);

(ii) a ligand capable of binding with a binding partner of the labeled component (a);

(iii) a ligand capable of binding with at least one of the component(s) to be determined; and

(iv) a ligand capable of binding with at least one of the binding partners of at least one of the component(s) to be determined; and

(c) directions for the performance of a protocol for the detection and/or determination of one or more components of an immunochemical reaction between the receptor recognition factor and a specific binding partner thereto.

In accordance with the above, an assay system for screening potential drugs effective to modulate the activity of the receptor recognition factor may be prepared. The receptor recognition factor may be introduced into a test system, and the prospective drug may also be introduced into the resulting cell culture, and the culture thereafter examined to observe any changes in the transcriptional activity of the cells, due either to the addition of the prospective drug alone, or due to the effect of added quantities of the known receptor recognition factor.

PRELIMINARY CONSIDERATIONS

As mentioned earlier, the observation and conclusion underlying the present invention were crystallized from a consideration of the results of certain investigations with particular stimuli. Particularly, the present disclosure is illustrated by the results of work on protein factors that govern transcriptional control of IFNα-stimulated genes, as well as more recent data on the regulation of transcription of genes stimulated by IFNγ. The following is a brief discussion of the role that IFN is believed to play in the stimulation of transcription taken from Darnell et al. *THE NEW BIOLOGIST*, 2(10), (1990).

Activation of genes by IFNα occurs within minutes of exposure of cells to this factor (Larner et al., 1984, 1986) and is strictly dependent on the IFNα binding to its receptor, a 49-kD plasma membrane polypeptide (Uze et al., 1990). However, changes in intracellular second messenger concentrations secondary to the use of phorbol esters, calcium ionophores, or cyclic nucleotide analogs neither triggers nor blocks IFNα-dependent gene activation (Larner et al., 1984; Lew et al., 1989). No other polypeptide, even IFNγ, induces the set of interferon-stimulated genes (ISGs) specifically induced by IFNα. In addition, it has been found that

IFNγ-dependent transcriptional stimulation of at least one gene in HeLa cells and in fibroblasts is also strictly dependent on receptor-ligand interaction and is not activated by induced changes in second messengers (Decker et al., 1989; Lew et al., 1989). These highly specific receptor-ligand interactions, as well as the precise transcriptional response, require the intracellular recognition of receptor occupation and the communication to the nucleus to be equally specific.

The activation of ISGs by IFNα is carried out by transcriptional factor ISGF-3, or interferon stimulated gene factor 3. This factor is activated promptly after IFNα treatment without protein synthesis, as is transcription itself (Larner et al., 1986; Levy et al., 1988; Levy et al., 1989). ISGF-3 binds to the ISRE, the interferon-stimulated response element, in DNA of the response genes (Reich et al., 1987; Levy et al., 1988), and this binding is affected by all of an extensive set of mutations that also affects the transcriptional function of the ISRE (Kessler et al., 1988a). Partially purified ISGF-3 containing no other DNA-binding components can stimulate ISRE-dependent *in vitro* transcription (Fu et al., 1990). IFN-dependent stimulation of ISGs occurs in a cycle, reaching a peak of 2 hours and declining promptly thereafter (Larner et al., 1986). ISGF-3 follows the same cycle (Levy et al., 1988, 1989). Finally, the presence or absence or ISGF3 in a variety of IFN-sensitive and IFN-resistant cells correlates with the transcription of ISGs in these cells (Kessler et al., 1988b).

ISGF-3 is composed of two subfractions, ISGF-3α and ISGF-3γ, that are found in the cytoplasm before IFN binds to its receptor (Levy et al., 1989). When cells are treated with IFNα, ISGF-3 can be detected in the cytoplasm within a minute, that is, some 3 to 4 minutes before any ISGF-3 is found in the nucleus (Levy et al., 1989). The cytoplasmic component ISGF-3γ can be increased in HeLa cells by pretreatment with IFNγ, but IFNγ does not by itself activate transcription of ISGs nor raise the concentration of the complete factor, ISGF-3 (Levy et al., 1990). The cytoplasmic localization of the proteins that interact to constitute ISGF-3 was proved by two kinds of experiments. When cytoplasm of IFNγ-treated cells that

lack ISGF-3 was mixed with cytoplasm of IFNα-treated cells, large amounts of ISGF-3 were formed (Levy et al., 1989). (It was this experiment that indicated the existence of an ISGF-3γ component and an ISGF-3α component of ISGF-3). In addition, Dale et al. (1989) showed that enucleated cells could respond to IFNα by forming a DNA-binding protein that is probably the same as ISGF-3.

The ISGF-3γ component is a 48-kD protein that specifically recognizes the ISRE (Kessler et al., 1990; Fu et al., 1990). Three other proteins, presumably constituting the ISGF-3α component, were found in an ISGF-3 DNA complex (Fu et al., 1990). The entirety of roles of, or the relationships among these three proteins are not yet known, but it is clear that ISGF-3 is a multimeric protein complex. Since the binding of IFNα to the cell surface converts ISGF-3α from an inactive to an active status within a minute, at least one of the proteins constituting ISGF-3α must be affected promptly, perhaps by a direct interaction with the IFNα receptor.

The details of how the ISGF-3γ component and the three other proteins are activated by cytoplasmic events and then enter the nucleus to bind the ISRE and increase transcription are not entirely known. Further studies of the individual proteins, for example, with antibodies, are presented herein. For example, it is clear that, within 10 minutes of IFNα treatment, there is more ISGF-3 in the nucleus than in the cytoplasm and that the complete factor has a much higher affinity for the ISRE than the 48-kD ISGF-3γ component by itself (Kessler et al., 1990).

In summary, the attachment of interferon-α (IFN-α) to its specific cell surface receptor activates the transcription of a limited set of genes, termed ISGs for "interferon stimulated genes" [Larner et al., *PROC. NATL. ACAD. SCI. USA*, **81** (1984); Larner et al., *J. BIOL. CHEM.*, **261** (1986); Friedman et al., *CELL*, **38** (1984)]). The observation that agents that affect second messenger levels do not activate transcription of these genes, led to the proposal that protein:protein

interactions in the cytoplasm beginning at the IFN receptor might act directly in transmitting to the nucleus the signal generated by receptor occupation [Levy et al., *NEW BIOLOGIST*, **2** (1991)].

To test this hypothesis, the present applicants began experiments in the nucleus at the activated genes. Initially, the ISRE and ISGF-3 were discovered [Levy et al., *GENES & DEV.*, **2** (1988)].

Partial purification of ISGF-3 followed by recovery of the purified proteins from a specific DNA-protein complex revealed that the complete complex was made up of four proteins [Fu et al., *PROC. NATL. ACAD. SCI. USA*, **87** (1990); Kessler et al., *GENES & DEV.*, **4** (1990)]. A 48 kD protein termed ISGF-3γ, because pre-treatment of HeLa cells with IFN-γ increased its presence, binds DNA weakly on its own [*Ibid.*; and Levy et al., *THE EMBO. J.*, **9** (1990)]. In combination with the IFN-α activated proteins, termed collectively the ISGF-3α proteins, the ISGF-3γ forms a complex that binds the ISRE with a 50-fold higher affinity [Kessler et al., *GENES & DEV.*, **4** (1990)]. The ISGF-3α proteins comprise a set of polypeptides of 113, 91 and 84 kD. All of the ISGF-3 components initially reside in the cell cytoplasm [Levy et al., *GENES & DEV.*, **3** (1989); Dale et al., *PROC. NATL. ACAD. SCI. USA*, **86** (1989)]. However after only about five minutes of IFN-α treatment the active complex is found in the cell nucleus, thus confirming these proteins as a possible specific link from an occupied receptor to a limited set of genes [Levy et al., *GENES & DEV.*, **3** (1989)].

In accordance with the present invention, specific proteins comprising receptor recognition factors have been isolated and sequenced. These proteins, their fragments, antibodies and other constructs and uses thereof, are contemplated and presented herein. To understand the mechanism of cytoplasmic activation of the ISGF-3α proteins as well as their transport to the nucleus and interaction with ISGF-3γ, this factor has been purified in sufficient quantity to obtain peptide sequence from each protein. Degenerate deoxyoligonucleotides that would encode

the peptides were constructed and used in a combination of cDNA library screening and PCR amplification of cDNA products copied from mRNA to identify cDNA clones encoding each of the four proteins. What follows in the examples presented herein a description of the final protein preparations that allowed the cloning of cDNAs encoding all the proteins, and the primary sequence of the 113 kD protein arising from a first gene, and the primary sequences of the 91 and 84 kD proteins which appear to arise from two differently processed RNA products from another gene. Antisera against portions of the 84 and 91 kD proteins have also been prepared and bind specifically to the ISGF-3 DNA binding factor (detected by the electrophoretic mobility shift assay with cell extracts) indicating that these cloned proteins are indeed part of ISGF-3. The availability of the cDNA and the proteins they encode provides the necessary material to understand how the liganded IFN-α receptor causes immediate cytoplasmic activation of the ISGF-3 protein complex, as well as to understand the mechanisms of action of the receptor recognition factors contemplated herein. The cloning of each of ISGF3-α proteins, and the evaluation and confirmation of the particular role played by the 91 kD protein as a messenger and DNA binding protein in response to IFN-γ activation, including the development and testing of antibodies to the receptor recognition factors of the present invention, are all presented in the examples that follow below.

EXAMPLE 1

To purify relatively large amounts of ISGF-3, HeLa cell nuclear extracts were prepared from cells treated overnight (16-18 h) with 0.5 ng/ml of IFN-γ and 45 min. with IFN-α (500u/ml). The steps used in the large scale purification were modified slightly from those described earlier in the identification of the four ISGF-3 proteins.

Accordingly, nuclear extracts were made from superinduced HeLa cells [Levy et al., *THE EMBO. J.*, **9** (1990)] and chromatographed as previously described [Fu

et al., *PROC. NATL. ACAD. SCI. USA*, **87** (1990)] on: phosphocellulose P-11, heparin agarose (Sigma); DNA cellulose (Boehringer Mannheim; flow through was collected after the material was adjusted to 0.28M KCl and 0.5% NP-40); two successive rounds of ISRE oligo affinity column (1.8 ml column, eluted with a linear gradient of 0.05 to 1.0M KCl); a point mutant ISRE oligonucleotide affinity column (flow through was collected after the material was adjusted to 0.28M KCl); and a final round on the ISRE oligonucleotide column (material was eluted in a linear 0.05 to 1.0M NaCl gradient adjusted to 0.05% NP-40). Column fractions containing ISGF-3 were subsequently examined for purity by SDS PAGE/silver staining and pooled appropriately. The pooled fractions were concentrated by a centricon-10 (Amicon). The pools of fractions from preparations 1 and 2 were combined and run on a 10 cm wide, 1.5 mm thick 7.5% SDS polyacrylamide gel. The proteins were electroblotted to nitrocellulose for 12 hrs at 20 volts in 12.5% MeOH, 25mM Tris, 190 mM glycine. The membrane was stained with 0.1% Ponceau Red (in 1% acetic acid) and the bands of 113 kD, 91 kD, 84 kD, and 48 kD excised and subjected to peptide analysis after tryptic digestion [Wedrychowski et al., *J. BIOL. CHEM.*, **265** (1990); Aebersold et al., *PROC. NATL. ACAD. SCI. USA*, **84** (1987)]. The resulting peptide sequences for the 91 kD and 84 kD proteins are indicated in Fig. 6. Degenerate oligonucleotides were designed based on the peptide sequences t19, t13b and t27: (Forward and Reverse complements are denoted by F and R:

19F AACGTIGACCAATTNAACATG;
T T GC T

T
13bR GTCGATGTTNGGGTANAG; 27R GTACAAITCAACCAGNGCAA
A A A A A T TG T T

The final ISRE oligonucleotide affinity selection yielded material with the SDS polyacrylamide gel electrophoretic pattern shown in Fig. 4 (left). This gel represented about 1.5% of the available material purified from over 200 L of appropriately treated HeLa cells. While 113, 91, 84 and 48 kD bands were

clearly prominent in the final purified preparation (see Fig. 4, right panel), there were also two prominent contaminants of about 118 and 70 kD and a few of other contaminants in lower amounts. [Amino acid sequence data have shown that the contaminants of 86 kD and 70 kD are the KU antigen, a widely-distributed protein that binds DNA termini. However in the specific ISGF-3: ISRE complex there is no KU antigen and therefore it has been assigned no role in IFN-dependent transcriptional stimulation, [Wedrychowski et al., *J. BIOL. CHEM.*, **265** (1990)]].

Since the mobility of the 113, 91, 84, and 48 kD proteins could be accurately marked by comparison with the partially purified proteins characterized in previous experiments [Fu et al., *PROC. NATL. ACAD. SCI. USA*, **87** (1990)], further purification was not attempted at this stage. The total purified sample from 200 L of HeLa cells was loaded onto one gel, subjected to electrophoresis, transferred to nitrocellulose and stained with Ponceau red. The 113, 84, 91, and 48 kD protein bands were separately excised and subjected to peptide analysis as described [Aebersold et al., *PROC. NATL. ACAD. SCI. USA*, **84** (1987)]. Released peptides were collected, separated by HPLC and analyzed for sequence content by automated Edman degradation analysis.

Accordingly, the use of the peptide sequence data for three of four peptides from the 91 kD protein and a single peptide derived from the 84 kD protein is described herein. The peptide sequence and the oligonucleotides constructed from them are given in the legend to Fig. 4 or 6. When oligonucleotides 19F and 13bR were used to prime synthesis from a HeLa cell cDNA library, a PCR product of 475 bp was generated. When this product was cloned and sequenced it encoded the 13a peptide internally. Oligonucleotide 27R derived from the only available 84 kD peptide sequence was used in an anchored PCR procedure amplifying a 405 bp segment of DNA. This 405 bp amplified sequence was identical to an already sequenced region of the 91 kD protein. It was then realized that the peptide t27 sequence was contained within peptide t19 and that the 91 and 84 kD proteins must be related (see Fig. 5 & 7). Oligonucleotides 19F and 13a were also used to

select candidate cDNA clones from a cDNA library made from mRNA prepared after 16 hr. of IFN-γ and 45 min. of IFN-α treatment.

Of the numerous cDNA clones that hybridized these oligonucleotides and also the cloned PCR products, one cDNA clone, E4, contained the largest open reading frame flanked by inframe stop codons. Sequence of peptides t19, t13a, and t13b were contained in this 2217 bp ORF (see Fig. 6) which was sufficient to encode a protein of 739 amino acids (calculated molecular weight of 86 kD). The codon for the indicated initial methionine was preceded by three in frame stop codons. This coding capacity has been confirmed by translating in vitro an RNA copy of the E4 clone yielding product of nominal size of 86 kD, somewhat shorter than the *in vitro* purified 91 kD protein discussed earlier (data not shown). Perhaps this result indicates post-translational modification of the protein in the cell.

A second class of clones was also identified (see Fig. 5). E3, the prototype of this class was identical to E4 from the 5' end to bp 2286 (aa 701) at which point the sequences diverged completely. Both cDNAs terminated with a poly(A) tail. Primer extension analysis suggested another ~150 bp were missing from the 5' end of both mRNAs. DNA probes were made from the clones representing both common and unique sequences for use in Northern blot analyses. The preparation of the probes is as follows: 20 mg of cytoplasmic RNA (0.5% NP-40 lysate) of IFN-α treated (6 h) HeLa RNA was fractionated in a 1% agarose, 6% formaldehyde gel (in 20 mM MOPS, 5mM NaAc, 1 mM EDTA, pH 7.0) for 4.5 h at 125 volts. The RNA was transferred in 20 x SSC to Hybond-N (Amersham), UV crosslinked and hybridized with 1x106 cpm/ml of the indicated probes ($1.5x10^8$ cpm/mg).

Probes from regions common to E3 and E4 hybridized to two RNA species of approximately 3.1 KB and 4.4 KB. Several probes derived from the 3' non-coding end of E4, which were unique to E4, hybridized only the larger RNA

species. A labeled DNA probe from the unique 3' non-coding end of E3 hybridized only the smaller RNA species.

Review of the sequence at the site of 3' discontinuity between E3 and E4 suggested that the shorter mRNA results from choice of a different poly(A) site and 3' exon that begins at bp 2286 (the calculated molecular weight from the E3. The last two nucleotides before the change are GT followed by GT in E3 in line with the consensus nucleotides at an exon-intron junction. Since the ORF of E4 extends to bp 2401 it encodes a protein that is 38 amino acids longer than the one encoded by E3, but is otherwise identical (ORF is 82 kD).

Since there is no direct assay for the activity of the 91 or 84 kD protein, an independent method was needed to determine whether the cDNA clones we had isolated did indeed encode proteins that are part of ISGF-3. For this purpose antibodies were initially raised against the sequence from amino acid 597 to amino acid 703 (see Fig. 6) by expressing this peptide in the pGEX-3X vector (15) as a bacterial fusion protein. This antiserum (a42) specifically recognized the 91 kD and 84 kD proteins in both crude extracts and purified ISGF-3 (see Fig. 7a). More importantly this antiserum specifically affected the ISGF-3 band in a mobility shift assay using the labeled ISRE oligonucleotide (see Fig. 7b) confirming that the isolated 91 kD and 84 kD cDNA clones (E4 and E3) represent a component of ISGF-3. Additional antisera were raised against the amino terminus and carboxy terminus of the protein encoded by E4. The amino terminal 59 amino acids that are common to both proteins and the unique carboxy terminal 34 amino acids encoded only by the larger mRNA were expressed as fusion proteins in pGEX-3X for immunization of rabbits. Western blot analysis with highly purified ISGF-3 demonstrated that the amino terminal antibody (a55) recognized both the 91 kD and 84 kD proteins as expected. However, the other antibody (a57) recognized only the 91 kD protein confirming our assumption that the larger mRNA (4.4 KB) and larger cDNA encodes the 91 kD protein while the shorter mRNA (3.1 KB) and cDNA encodes the 84 kD protein (see Fig. 7a).

EXAMPLE 2

In this example, the cloning of the 113 kD protein that comprises one of the three ISGF-3α components is disclosed.

From SDS gels of highly purified ISGF-3, the 113 kD band was identified, excised and subjected to cleavage and peptide sequence analysis [Aebersold et al., *PROC. NATL. ACAD. SCI. USA*, **87** (1987)]. Five peptide sequences (A-E) were obtained (Fig. 8A). Degenerate oligonucleotide probes were designed according to these peptides which then were radiolabeled to search a human cDNA library for clones that might encode the 113 kD protein. Eighteen positive cDNA clones were recovered from 2.5 x 10^5 phage plaques with the probe derived from peptide E (Fig. 8A, and the legend). Two of them were completely sequenced. Clone f11 contained a 3.2 KB cDNA, and clone ka31 a 2.6 KB cDNA that overlapped about 2 KB but which had a further extended 5' end in which a candidate AUG initiation codon was found associated with a well-conserved Kozak sequence [Kozak, *NUCLEIC ACIDS RES.*, **12** (1984)].

In addition to the phage cDNA clones, a PCR product made between oligonucleotides that encoded peptide D and E also yielded a 474 NT fragment that when sequenced was identical with the cDNA clone in this region. A combination of these clones f11 and ka31 revealed an open reading frame capable of encoding a polypeptide of 851 amino acids (Fig. 8A). These two clones were joined within their overlapping region and RNA transcribed from this recombinant clone was translated in vitro yielding a polypeptide that migrated in an SDS gel with a nominal molecular weight of 105 kD (Fig. 9A). An appropriate clone encoding the 91 kD protein was also transcribed and the RNA translated in the same experiment. Since both the apparently complete cDNA clones for the 113 kD protein and the 91 kD protein produce RNAs that when translated into proteins migrate somewhat faster than the proteins purified as ISGF-3 components, it is possible that the proteins undergo post-translational modification in the cell causing

them to be slightly retarded during electrophoresis. When a 660 bp cDNA encoding the most 3' end of the 113 kD protein was used in a Northern analysis, a single 4.8 KB mRNA species was observed (Figure 9B).

No independent assay is known for the activity of the 113 kD (or indeed any of the ISGF-3α proteins,) but it is known that the protein is part of a DNA binding complex that can be detected by an electrophoretic mobility shift assay [Fu et al., *PROC. NATL. ACAD. SCI. USA*, **87** (1990)]. Antibodies to DNA binding proteins are known to affect the formation or migration of such complexes. Therefore antiserum to a polypeptide segment (amino acid residues 323 to 527) fused with bacterial glutathione synthetase [Smith et al., *PROC. NATL. ACAD. SCI. USA*, **83** (1986)] was raised in rabbits to determine the reactivity of the ISGF-3 proteins with the antibody. A Western blot analysis showed that the antiserum reacted predominantly with a 113 kD protein both in the ISGF3 fraction purified by specific DNA affinity chromatography (Lane 1) and in crude cell extract (Lane 2, Fig. 10A). The weak reactivity to lower protein bands was possibly due to 113 kD protein degradation. Most importantly, the antiserum specifically removed almost all of the gel-shift complex leaving some of the oligonucleotide probe in "shifted-shift" complexes which were specifically competed away with a 50 fold molar excess of the oligonucleotide binding site (the ISRE, ref. 2) for ISGF3 (Fig. 10B). Notably, this antiserum had no effect on the faster migrating shift band produced by ISGF3-γ component alone (Figure 10B). Thus it appeared that the antiserum to the 113 kD fusion product does indeed react with another protein that is part of the complete ISGF-3 complex.

A detailed sequence comparison between the 113 and 91 sequences followed (Fig. 8B): while the nucleotide sequence showed only a distant relationship between the two proteins, there were long stretches of amino acid identity. These conserved regions were scattered throughout almost the entire 715 amino acid length encoded by the 91/84 clone. It was particularly striking that the regions corresponding to amino acids 1 to 48 and 317 to 353 and 654 to 678 in the 113 sequence were 60%

to 70% identical to corresponding regions of the 91 kD sequence. Thus the genes encoding the 113 and 84/91 proteins are closely related but not identical.

Through examination for possible consensus sequences that might reveal sub-domain structures in the 113 kD or 84/91 kD sequence, it was found that both proteins contained regions whose sequence might form a coil structure with heptad leucine repeats. This occurred between amino acid 210 and 245 in the 113 kD protein and between 209 and 237 in the 84/91 protein. In both the 113 kD and the 91/84 kD sequences, 4 out of 5 possible heptad repeats were leucine and one was valine. Domains of this type might provide a protein surface that encourages homo-or heterotypic protein interactions which have been observed in several other transcription factors [Vinson et al., *SCIENCE*, **246** (1989)]. An extended acidic domain was located at the carboxyl terminal of the 113 kD protein but not in 91 kD protein (Fig. 8A), possibly implicating the 113 kD protein in gene activation [Hope et al., Ma et al., *CELL*, **48** (1987)].

DISCUSSION

When compared at moderate or high stringency to the Genbank and EMBL data bases, there were no sequences like 113 or the 84/91 sequence. Preliminary PCR experiments however indicate that there are other family members with different sequences recoverable from a human cell cDNA library (Qureshi and Darnell unpublished). Thus, it appears that the 113 and 84/91 sequences may represent the first two members to be cloned of a larger family of proteins. We would hypothesize that the 113 kD and 84/91 kD proteins may act as signal transducers, somehow interacting with the internal domain of a liganded IFNα receptor or its associated protein and further that a family of waiting cytoplasmic proteins exist whose purpose is to be specific signal transducers when different receptors are occupied. Many experiments lie ahead before this general hypothesis can be crucially tested. Recent experiments have indicated that inhibitors of protein kinases can prevent ISGF-3 complex formulation [Reich et al., *PROC. NATL. ACAD. SCI. USA*, **87** (1990); Kessler et al., *J. BIOL. CHEM.*, **266** (1991)].

However, neither the IFNα or IFNγ receptors that have so far been cloned have intrinsic kinase activity [Uze et al., *CELL*, **60** (1990); Aguet et al., *CELL*, **55** (1988)]. We would speculate that either a second receptor chain with kinase activity or a separate kinase bound to a liganded receptor could be a part of a complex that would convey signals to the ISGF-3α proteins at the inner surface of the plasma membrane.

From the above, it has been concluded that accurate peptide sequence from ISGF-3 protein components have been determined, leading to correct identification of cDNA clones encoding the 113, 91 and 84 kD components of ISGF-3 . Since staurosporine, a broadly effective kinase inhibitor blocks IFN-α induction of transcription and of ISGF-3 formation [Reich et al., *PROC. NATL. ACAD. SCI. USA*, **87** (1990); Kessler et al., *J. BIOL. CHEM.*, **266** (1991)] it seems possible that the ISGF-3α proteins are direct cytoplasmic substrates of a liganded receptor-associated kinase. The antiserum against these proteins should prove invaluable in identifying the state of the ISGF-3α proteins before and after IFN treatment and will allow the direct exploration of the biochemistry of signal transduction from the IFN receptor.

EXAMPLE 3

As mentioned earlier, the observation and conclusion underlying the present invention were crystallized from a consideration of the results of certain investigations with particular stimuli. Particularly, the present disclosure is illustrated by the results of work on protein factors that govern transcriptional control of IFNα-stimulated genes, as well as more recent data on the regulation of transcription of genes stimulated by IFNγ.

For example, there is evidence that the 91 kD protein is the tyrosine kinase target when IFNγ is the ligand. Thus two different ligands acting through two different receptors both use these family members. With only a modest number of family

members and combinatorial use in response to different ligands, this family of proteins becomes an even more likely possibility to represent a general link between ligand-occupied receptors and transcriptional control of specific genes in the nucleus.

Further study of the 113, 91 and 84 kD proteins of the present invention has revealed that they are phosphorylated in response to treatment of cells with IFNα (Figure 11). Moreover, when the phosphoamino acid is determined in the newly phosphorylated protein the amino acid has been found to be tyrosine (Fig. 12). This phosphorylation has been observed to disappear after several hours, indicating action of a phosphatase of the 113, 91 and 84 kD proteins to stop transcription. These results show that IFN dependent transcription very likely demands this particular phosphorylation and a cycle of interferon-dependent phosphorylation-dephosphorylation is responsible for controlling transcription.

It is proposed that other members of the 113-91 protein family will be identified as phosphorylation targets in response to other ligands. If as is believed, the tyrosine phosphorylation site on proteins in this family is conserved, one can then easily determine which family members are activated (phosphorylated), and likewise the particular extracellular polypeptide ligand to which that family member is responding. The modifications of these proteins (phosphorylation and dephosphorylation) enables the preparation and use of assays for determining the effectiveness of pharmaceuticals in potentiating or preventing intracellular responses to various polypeptides, and such assays are accordingly contemplated within the scope of the present invention.

EXAMPLE 4

In previous experiments, an exonuclease protection assay identified an IFN-γ dependent, site-specific DNA binding protein, termed GAF [gamma activating factor (10)]. The DNA site on which this complex specifically formed was termed

GAS (10,14). A protein capable of forming an electrophoretically stable DNA-protein complex with the same characteristics as GAF has now been identified in extracts of fibroblasts, by the much more convenient electrophoretic mobility shift assay (16) (Fig. 13). The gel-shift complex is induced in 15 min. by IFN-γ but not IFN-α (Fig. 13A, lanes 1-3) and is specifically competed by the GAS oligonucleotide and not by the ISRE (3) which is the IFN-α responsive site (Fig. 13A, lanes 5-6). The prompt IFN-γ dependent activation of this factor occurs without new protein synthesis (Fig. 13B and 13C). This DNA binding activity appears within minutes of IFN-γ treatment, is maximal between 15 and 30 minutes and then disappears after 2 to 3 hours (Fig. 13C) which correlates with the time course of INF-γ induction of the GBP gene in fibroblasts (9,10). Thus, the factor assayed by electrophoretic mobility shift assay has the same behavior as the factor previously described using the exonuclease III assay. The factor producing the gel shift is therefore referred to as GAF (the gamma activating factor).

EXAMPLE 5

A 91 kD protein contacts DNA

To test the size of the protein or proteins that contact DNA in the GAF, an experiment which crosslinked protein to the GAS oligonucleotide was carried out. N_3dUTP substituted, ^{32}P labeled GAS oligonucleotide was mixed with extracts of fibroblasts which had been treated with IFN-γ. The DNA complex was identified by gel retardation and autoradiography after which the gel was exposed to UV irradiation. The GAF shift band was cut out and subjected to SDS gel electrophoresis. A single band that migrated at 97 kD was observed. The protein in the oligonucleotide-protein complex therefore appeared to be in the $\approx$90 kD range, the same size range as one of the ISGF-3 proteins (7,12).

Although it is known that IFN-α and IFN-γ induce factors that recognized different DNA binding sites, both ligands produce the anti-viral state and arrest

cell growth and both induce some of the overlapping genes (2,17). Therefore with the availability of anti-sera (12) to ISGF-3α proteins (the 113, 91 and 84 kD proteins activated by IFN-α) and the knowledge that a 91 kD protein could be visualized binding to the GAS site (Fig. 14A), the possible effect of the antisera to 113 kD and 91 kD proteins on the GAF gel-shift was tested (Fig. 14B). Two sera against segments of the 91 kD protein were available (12), one to amino acids 597 to 703 which recognizes the 91 and 84 kD protein component of ISGF-3 and a second antibody to the terminal 36 amino acids that are present in the 91 kD protein but are absent from the 84 kD protein (12). In addition antisera to the 48 and 113 kD proteins were also available.

When the specific GAF gel-shift complex was tested, it was found that the antiserum to the center section of the 91 kD protein produced a greatly retarded ("supershift") band and the serum to the carboxyl terminus of the 91 kD protein which does not recognize the 84 kD protein blocked the formation of the IFN-γ specific gel shift complex (Fig. 14B, lanes 3-6). This gel shift complex was not affected by antisera against the 113 kD or 48 kD proteins (data not shown). All of these experiments suggest that the 91 kD protein contacts DNA and participates in the GAF gel-shift complex while the other ISGF-3 proteins do not.

EXAMPLE 6

Only the 91 kD protein can be found in GAF

To further characterize the protein in the IFN-γ dependent gel shift complex, INF-γ treated extracts were subjected to one step purification by adsorption to a biotinylated GAS oligonucleotide (18). To directly identify the size of the protein in the GAF gel-shift complex, the affinity purified fraction was analyzed by two-dimensional gel mobility shift-SDS electrophoresis. The DNA binding reaction was performed with ^{32}P labeled oligonucleotide and unlabeled protein and mobility shift gel (16) was used to separate out the GAF complex (Fig. 14C). The position of the GAF band was identified by autoradiography and the gel lane was rotated

90°, and subjected to electrophoresis in an SDS acrylamide gel. After SDS polyacrylamide gel electrophoresis, the gel was electroblotted onto nitrocellulose and the constituent proteins tested with antiserum to the 91 kD protein by immunoblot analysis (ECL kit, Amersham).

The 91 kD protein was indeed found to be detected in the shift complex by the antiserum specific to the 91 kD protein. Reprobing of the same blots with antisera against the 113 kD or the 48 kD proteins indicated that they were not present in the GAF shift complex (data not shown). In this experiment the initial DNA binding reaction was dependent on the GAS oligonucleotide; no gel shift complex was observed without the GAS oligonucleotide and no immunoreactive protein was recovered from the position of the gel-shift complex in parallel samples run without the DNA binding site.

In an attempt to identify any other proteins in the GAF complex, cells were labeled with ^{35}S methionine for fourteen hours, extracts prepared and subjected to affinity purification using the biotinylated GAS oligonucleotide as described for Fig. 14C. ^{35}S labeled proteins eluted from the biotinylated oligonucleotide complex were then used in a gel shift reaction containing ^{32}P labeled GAS oligonucleotide to locate the GAF band by autoradiography as had been done earlier with unlabeled proteins (Fig. 14D). A similar reaction was also run with antiserum to the 91 kD protein included in the binding reactions to block the GAF complex formation and the two reactions were analyzed in parallel. After locating the GAF complex, the two gel lanes were rotated 90° as described before and subjected to a second SDS gel electrophoresis to separate individual proteins according to size.

Autoradiography showed a number of ^{35}S labeled proteins were recovered from both gel lanes that were not in the position of the gel-shift complex, and only one distinctly labeled protein, the 91 kD protein, was present in the region of the specific gel shift. Furthermore, the 91 kD protein was not present (in that

postion) if treatment with the 91 kD antibody was carried out during complex formation. If the 113, 84 or 48 kD proteins or indeeed any other specific protein had been present in the GAF complex in amounts that would be near stoichiometric with the 91 kD protein, they should have been visualized since the treated cells had been labeled for 14 hours. Only if a protein lacked or had a very low methionine content would it not have been detected. Thus the experiments in Fig. 13 support the possibility that the 91 kD protein which can be cross-linked by UV irradiation to the GAF oligonucleotide, is the sole protein in the GAF complex.

EXAMPLE 7

Translocation of the 91 kD protein to the nucleus after IFN-γ treatment

Fluorescent antibody tests were next used to examine the cellular localization of the 113, 91 and 84 kD proteins after IFN-γ treatment (Fig. 15). Antisera against the 113 kD protein showed a generalized cellular fluorescence with no reaction in the nucleus and no change after IFN-γ treatment (Fig. 14C, 14D). By contrast, antiserum specific for the COOH-terminal amino acids of the 91 kD protein that are not contained in the 84 kD protein, showed intense nuclear fluorescence within minutes of IFN-γ treatment (Figs. 15A, 15B).

Thus the 91 kD but not the 113 kD protein is promptly translocated to the nucleus after IFN-γ treatment, while all three proteins 113, 91 and 84 are translocated to the nucleus after IFN-α treatment (12,15). Without an antiserum specific to the 84 kD protein which is entirely contained within the 91 kD protein, it cannot be determined by antiserum alone whether the 84 kD protein participates in INF-α activation, however as shown in Fig. 14, the 84 kD protein was not found in the GAF.

EXAMPLE 8

Evidence for phosphorylation in the activation of the 91 kD protein

It was next determined to test the nature of any changes in the 91 kD protein after IFN-γ treatment by careful analysis of the migration of 91 kD protein both before and after treatment. First Western blots showed that IFN-γ treatment induced a slower migrating form of the 91 kD protein on SDS gel electrophoresis, while only the faster migrating form could be identified in untreated extracts (Fig. 16A). The presence of the slower migrating form of the 91 kD protein paralleled in time the presence of the GAF DNA binding activity (Fig. 16A and 13C) being maximal at 15 min. of treatment and gone by 2 hours of treatment. Only the slower migrating form could be detected in the affinity purified fraction of GAF indicating that only the slower migrating protein had high DNA binding affinity (Fig. 16B).

Phosphorylation would produce a slower electrophoretic migration, and therefore affinity purified GAF was treated with calf intestinal phosphatase, the samples were electrophoresed and thereafter assayed for the 91 kD protein by Western blot (Fig. 16B). The slower migrating form was converted to the faster migrating form by phosphatase treatment. Finally, an inhibitor of protein kinases, staurosporine, which blocks the IFN-α dependent phosphorylation of the 91 kD protein (15,19,20) was found to inhibit the IFN-γ induced phosphorylation. Both phosphatase treatment and staurosporine were found to block the GAF DNA binding activity (Fig. 16C). These data further support that the slower migrating form is the active form of GAF. It has also been found (data not shown) that staurosporine like H7 (8), another kinase inhibitor, will block the IFN-γ dependent transcriptlon of the GBP gene.

EXAMPLE 6

IFN-γ dependent ^{32}P tyrosine phosphorylation in 91 kD protein

Direct testing was next conducted for IFN-γ-dependent phosphorylation of the 91 kD protein. Cells were labeled with $^{32}PO_4$ and treated with IFN-γ. Extracts were

prepared and precipitated with a 91 kD antiserum, and immunoprecipitates were analyzed on SDS PAGE (Fig. 17A). There was indeed an IFN-γ dependent ^{32}P labeling of a 91 kD antiserum precipitable band. The electrophoretic migration of the ^{32}P labeled band corresponded to the slower migrating form of ^{35}S labeled 91 kD immunoprecipitate, while the band from untreated cells corresponded to the faster migrating form.

The ^{32}P labeled bands were recovered and cleaved by thermolysin treatment under conditions that yield small peptides. A fraction of each sample was used for phosphoamino acid analysis. While phosphotyrosine could not be detected in untreated cells, phosphotyrosine was strongly labeled in IFN-γ treated cells (Fig. 17B). Similar analyses were carried out with 113 kD protein and no phosphotyrosine was induced in that protein in response to IFN-γ (data not shown).

To further characterize the phosphorylation of 91 kD protein two-dimensional peptide mapping of thermolysin digests was performed. One very highly labeled phosphopeptide, X, detected only in IFN-γ treated cells and three less highly labeled phosphopeptides, a, b and c, detected in both treated and untreated cells, were observed (Fig. 18A, 18E). Each phosphopeptide was eluted and subjected to phosphoamino acid analysis. Only phosphotyrosine could be detected in peptide X while peptides a, b and c were found to contain only phosphoserine.

To confirm that tyrosine phosphorylation is a cytoplasmic event, 91 kD protein was immunoprecipitated from cytoplasmic extracts of cells that had been with IFN-γ for only 3 min. The same phosphotyrosine containing peptide X was detected by thermolysin peptide mapping of cytoplasmic fractions (Fig. 18D). Staurosporine, the protein kinase inhibitor, was found to inhibit IFN-γ induced phosphorylation in the 91 kD protein (Fig. 17A). Peptide mapping indicated that this inhibition was specifically on peptide X that bears the IFN-γ dependent tyrosine phosphate (Fig. 18B). Taken together with the fact that staurosporine

blocks the GAF DNA binding activity (Fig. 16C), it was concluded that the IFN-γ induced tyrosine phosphorylation on the 91 kD protein is required for the protein to bind to the GAS sequence and to activate transcription.

DISCUSSION

As mentioned earlier, the observation and conclusion underlying the present invention were crystallized from a consideration of the results of certain investigations with particular stimuli. Particularly, the present disclosure is illustrated by the results of work on protein factors that govern transcriptional control of IFNα-stimulated genes, as well as more recent data on the regulation of transcription of genes stimulated by IFNγ.

For example, the above represents evidence that the 91 kD protein is the tyrosine kinase target when IFNγ is the ligand. Thus two different ligands acting through two different receptors both use these family members. With only a modest number of family members and combinatorial use in response to different ligands, this family of proteins becomes an even more likely possibility to represent a general link between ligand-occupied receptors and transcriptional control of specific genes in the nucleus.

It is proposed that other members of the 113-91 protein family will be identified as phosphorylation targets in response to other ligands. If as is believed, the tyrosine phosphorylation site on proteins in this family is conserved, one can then easily determine which family members are activated (phosphorylated), and likewise the particular extracellular polypeptide ligand to which that family member is responding. The modifications of these proteins (phosphorylation and dephosphorylation) enables the preparation and use of assays for determining the effectiveness of pharmaceuticals in potentiating or preventing intracellular responses to various polypeptides, and such assays are accordingly contemplated within the scope of the present invention.

Earlier work has concluded that DNA binding protein was activated in the cell cytoplasm in response to IFN-γ treatment and that this protein stimulated transcription of the GBP gene (10,14). In the present work, with the aid of antisera to proteins originally studied in connection with IFN-α gene stimulation (7,12,15), the 91 kD ISGF-3 protein has been assigned a prominent role in IFN-γ gene stimulation as well. The evidence for this conclusion included: 1) antisera specific to the 91 kD proteln affected the IFN-γ dependent gel-shift complex, and 2) A 91 kD protein could be cross-linked to the GAS IFN-γ activated site. 3) A ^{35}S-labeled 91 kD protein and a 91 kD immunoreactive protein specifically purified with the gel-shift complex. 4) The 91 kD protein is an IFN-γ dependent tyrosine kinase substrate as indeed it had earlier proved to be in response to IFN-α (15). 5) The 91 kD protein but not the 113 kD protein moved to the nucleus in response to IFN-γ treatment. None of these experiments prove but do strongly suggest that the same 91 kD protein acts differently in different DNA binding complexes that are triggered by either IFN-α or IFN-γ.

These results strongly support the hypothesis originated from studies on IFN-α that polypeptide cell surface receptors report their occupation by extracellular ligand to latent cytoplasmic proteins that after activation move to the nucleus to trigger transcription (4,15,21). Furthermore, because cytoplasmic phosphorylation and factor activation is so rapid it appears likely that the functional receptor complexes contain tyrosine kinase activity. Since the IFN-γ receptor chain that has been cloned thus far (22) has no hint of possessing intrinsic kinase activity, perhaps some other molecule with tyrosine kinase activity couples with the IFN-γ receptor. Two recent results with other receptors suggest possible parallels to the situation with the IFN receptors. The *trk* protein which has an intracellular tyrosine kinase domain, associates with the NGF receptor when that receptor is occupied (23). In addition, the *lck* protein, a member of the *src* family of tyrosine kinases, is co-precipitated with the T cell receptor (24). It is possible to predict that signal transduction to the nucleus through these two receptors could involve latent cytoplasmic substrates that form part of activated transcription factors. In any

event, it seems possible that there are kinases like *trk* or *lck* associated with the IFN-γ receptor or with IFN-α receptor.

With regard to the effect of phosphorylation on the 91 kD protein, it was something of a surprise that after IFN-γ treatment the 91 kD protein becomes a DNA binding protein. Its role must be different in response to IFN-α treatment. There it is also phosphorylated on tyrosine and joins a complex with the 113 and 84 kD proteins but as judged by UV cross-linking studies (7), the 91 kD protein does not contact DNA.

In addition to becoming a DNA binding protein it is clear that the 91 kD protein is specifically translocated the nucleus in the wake of IFN-γ stimulation. While the present work strongly implicates the 91 kD protein as important in the immediate IFN-γ transcriptional response of the GBP gene, two points should also be clear. First, it is not known whether the 91 kD protein acts on its own to activate transcription. Second, it is not known how widely used the 91 kD protein is in the immediate IFN-γ transcriptional response. Only a few genes have been studied that are activated immediately by IFN-γ without new protein synthesis. It is at present uncertain whether activation of these genes operates through the 91 kD binding site.

Proof of the model that the 91 kD protein recognizes the liganded receptor demands proof of association with and/or phosphorylation by a membrane-associated receptor complex. To that end antibodies against the IFN-γ receptor chain have been prepared and attempts are underway to test this proposal. Since any potential substrate molecule would not be expected to dwell long at an activation site, it is not expected that most of the 91 kD protein will be associated with receptor complexes at any one time. Membrane associated kinase activity that is active at a critical site in the 91 kD protein, also yet to be definitely determined, could be the earliest indication that the proposal is correct.

The following is a list of references related to the above disclosure and particularly to the experimental procedures and discussions. The references are numbered to correspond to like number references that appear hereinabove.

1. Larner, A. C., Jonak, G., Cheng, Y. S., Korant, B., Knight, E. and Darnell, J. E., Jr. (1984). *Proc. Natl. Acad. Sci. USA* **81**:6733-6737; Larner, A. C., Chaudhuri, A. and Darnell, J. E. (1986). *J. Biol. Chem.* **261**:453-459.

2. Friedman, R. L., Manly, S. P., McMahon, M., Kerr, I. M. and Stark, G. R. (1984). *Cell* 38:745-755.

3. Levy, D. E., Kessler, D. S., Pine, R., Reich, N. and Darnell, J. E. (1988). *Genes & Dev.* **2**:383-392.

4. Levy, D. E., Kessler, D. S., Pine, R., and Darnell, J. E. (1989). *Genes & Dev.* **3**:1362-1371.

5. Dale, T. C., Iman, A. M. A., Kerr, I. M. and Stark, G. R. (1989). *Proc. Natl. Acad. Sci.* **86**:1203-1207.

6. Kessler, D. S., Veals, S. A., Fu, X.-Y., and Levy, D. E. (1990). *Genes & Dev.* **4**:1753-1765.

7. Fu, X. -Y., Kessler, D. S., Veals, S. A., Levy, D. E. and Darnell, J. E. (1990). *Proc. Natl. Acad. Sci. USA* **87**:8555-8559.

8. Lew, D. J., Decker, T., and Darnell, J. E. (1989). *Mol. Cell. Biol.* **9**:5404-5411.

9. Decker, T., Lew, D. J., Cheng, Y.-S., Levy, D. E. and Darnell, J. E. (1989). *EMBO J.* **8**:2009-2014.

10. Decker, T., Lew, D. J., Mirkovitch, J. and Darnell, J. E., 1991. *EMBO J.* **10**:927-932.

11. Veals, S. A., Schindler, C. W., Fu, X.-Y., Leonard, D., Darnell, J. E. and Levy, D. E. (1992). *Mol. Cell. Biol.* **12**, in press.

12. Schindler, C., Fu, X.-Y., Improta, T., Aebersold, R. and Darnell, J. E. (1992). *Proc. Natl. Acad. Sci. USA* **89**, in press.

13. Fu, X.-Y., Schindler, C., Improta, T., Aebersold, R. and Darnell, J. E. (1992). *Proc. Natl. Acad. Sci. USA* **89**, in press.

14. Lew, D. J., Decker, T. and Darnell, J. E. (1991). *Mol. Cell. Biol.* **11**:182-191.

15. Schindler, C., Shuai, K., Fu, X.-Y., Prezioso, V. and Darnell, J. E. (1992). *Science* **257**:809-812.

16. Garner, M. M. and Revan, A. (1981). *Nuc. Acids Res.* **9**:3047-3059; Fried, A., and Crothers, D. M. (1981) *ibid* 6505-6525.

17. Celis, J. E., Justessen, J., Madsun, P. S., Lovmand, J., Ratz, G. P. and Celis, A. (1987). *Leukemia* **1**:800-813.

18. Chodosh, L. A., Carthew, R. W. and Sharp, P. A. (1986). *Mol. Cell Biol.* **6**:4723-4733.

19. Reich, N. and Pfeffer, L. M. (1990). *Proc. Natl. Acad. Sci. USA* **87**:8761-8765.

20. Kessler, D. S. and Levy, D. (1991). *J. Biol. Chem.* **266**: 23471-23476.

21. Levy, D., and Darnell, J. E. (1990). *The New Biologist* **2**:923-928.

22. Aguet, J. M., Denbie, Z. and Merlin, G. (1986). *Cell* **55**:273-280.

23. Kaplan, D. R., Martin-Zanca, D. and Parada, L. F. (1991). *Nature* **350**:158-160; Hempstead, G., Kapland, D., Martin-Zanca, D., Parada, L. F. and Chao, M. (1991). *Nature* **350**:678-683.

24. Veillette, A., Bookman, M. A., Horak, E. M., and Bolen, J. B. (1988). *Cell* **55**:301-308; Rudd, C. E. et al. (1988). *Proc. Natl. Acad. Sci. USA* **85**:5190-5194.

25. Evans, R. K., Johnson, J. D. and Haley, B. E. (1986). *Proc. Natl. Acad. Sci. USA* **83**:5382-5386.

26. Walaas, S. I. and Nairn, A. C. (1989). *J. of Mol. Neurosci.* **1**:117-127.

This invention may be embodied in other forms or carried out in other ways without departing from the spirit or essential characteristics thereof. The present disclosure is therefore to be considered as in all respects illustrative and not restrictive, the scope of the invention being indicated by the appended Claims, and all changes which come within the meaning and range of equivalency are intended to be embraced therein.

WHAT IS CLAIMED IS:

1. A receptor recognition factor implicated in the transcriptional stimulation of genes in target cells in response to the binding of a specific polypeptide ligand to its cellular receptor on said target cell, said receptor recognition factor having the following characteristics:

a) apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene;

b) an activity demonstrably unaffected by the presence or concentration of second messengers;

c) direct interaction with tyrosine kinase domains; and

d) a perceived absence of interaction with G-proteins.

2. The receptor recognition factor of Claim 1, having the following characteristics:

a) It interacts with an interferon-γ-bound receptor kinase complex;

b) It is a tyrosine kinase substrate; and

c) When phosphorylated, it serves as a DNA binding protein.

3. The receptor recognition factor of Claim 2 further characterized in that:

d) Interferon-γ-dependent activation of said factor occurs without new protein synthesis; and

e) Activation of said factor appears within minutes of interferon-γ treatment, achieves maximum extent between 15 and 30 minutes thereafter, and then disappears after 2-3 hours.

4. The receptor recognition factor of Claim 1 which is proteinaceous in composition.

5. The receptor recognition factor of Claim 1 which is cytoplasmic in origin.

6. The receptor recognition factor of Claim 1 which is derived from mammalian cells.

7. The receptor recognition factor of Claim 1 labeled with a detectable label.

8. The receptor recognition factor of Claim 7 wherein the label is selected from enzymes, chemicals which fluoresce and radioactive elements.

9. An antibody to a receptor recognition factor, the factor to which said antibody is raised having the following characteristics:

a) apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene;

b) an activity demonstrably unaffected by the presence or concentration of second messengers; and

c) direct interaction with tyrosine kinase domains; and

d) a perceived absence of interaction with G-proteins.

10. The antibody of Claim 9, wherein said receptor recognition factor has the following characteristics:

a) It interacts with an interferon-γ-bound receptor kinase complex;

b) It is a tyrosine kinase substrate; and

c) When phosphorylated, it serves as a DNA binding protein.

11. The antibody of Claim 10, wherein said receptor recognition factor is further characterized in that:

d) Interferon-γ-dependent activation of said factor occurs without new protein synthesis; and

e) Activation of said factor appears within minutes of interferon-γ treatment, achieves maximum extent between 15 and 30 minutes thereafter, and then disappears after 2-3 hours.

12. The antibody of Claim 9 comprising a polyclonal antibody.

13. The antibody of Claim 9 comprising a monoclonal antibody.

14. An immortal cell line that produces a monoclonal antibody according to Claim 13.

15. The antibody of Claim 9 labeled with a detectable label.

16. The antibody of Claim 15 wherein the label is selected from enzymes, chemicals which fluoresce and radioactive elements.

17. A DNA sequence or degenerate variant thereof, which encodes a receptor recognition factor, or a fragment thereof, selected from the group consisting of:

(A) the DNA sequence of FIGURE 1;

(B) the DNA sequence of FIGURE 2;

(C) the DNA sequence of FIGURE 3;

(D) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions; and

(E) DNA sequences that code on expression for an amino acid sequence encoded by any of the foregoing DNA sequences.

18. A recombinant DNA molecule comprising a DNA sequence or degenerate variant thereof, which encodes a receptor recognition factor, or a fragment thereof, selected from the group consisting of:

(A) the DNA sequence of FIGURE 1;

(B) the DNA sequence of FIGURE 2;

(C) the DNA sequence of FIGURE 3;

(D) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions; and

(E) DNA sequences that code on expression for an amino acid sequence encoded by any of the foregoing DNA sequences.

19. The recombinant DNA molecule of either of Claims 17 or 18, wherein said DNA sequence is operatively linked to an expression control sequence.

20. The recombinant DNA molecule of Claim 19, wherein said expression control sequence is selected from the group consisting of the early or late promoters of SV40, CMV, vaccinia, polyoma or adenovirus, the *lac* system, the *trp* system, the *TAC* system, the *TRC* system, the *LTR* system, the major operator and promoter regions of phage λ, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase and the promoters of the yeast α-mating factors.

21. A probe capable of screening for the receptor recognition factor in alternate species prepared from the DNA sequence of Claim 17.

22. A unicellular host transformed with a recombinant DNA molecule comprising a DNA sequence or degenerate variant thereof, which encodes a receptor recognition factor, or a fragment thereof, selected from the group consisting of:

(A) the DNA sequence of FIGURE 1;

(B) the DNA sequence of FIGURE 2;

(C) the DNA sequence of FIGURE 3;

(D) DNA sequences that hybridize to any of the foregoing DNA sequences under standard hybridization conditions; and

(E) DNA sequences that code on expression for an amino acid sequence encoded by any of the foregoing DNA sequences;

wherein said DNA sequence is operatively linked to an expression control sequence.

23. The unicellular host of Claim 22 wherein the unicellular host is selected from the group consisting of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, yeasts, CHO, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, and BMT10 cells, plant cells, insect cells, and human cells in tissue culture.

24. A method for measuring the presence of a receptor recognition factor, said receptor recognition factor having the following characteristics: apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene; an activity demonstrably unaffected by the presence or concentration of second messengers; direct interaction with tyrosine kinase domains; and a perceived absence of interaction with G-proteins, wherein said receptor recognition factor is measured by:

A. preparing at least one sample of said receptor recognition factor;

B. preparing at least one corresponding antibody or binding partner directed to said receptor recognition factor samples;

C. placing a detectible label on a material selected from the group consisting of said receptor recognition factor samples and said antibody or binding partners thereto;

D. immobilizing a material selected from the group consisting of the material from Step C that is not labeled, and a biological sample from a mammal in which the presence and/or activity of said receptor recognition factor is suspected, on a suitable substrate;

E. placing the labeled material from Step C in contact with said biological sample, and in contact with the immobilized material;

F. separating the material from Step C that is bound to said immobilized material from material from Step C not bound to said immobilized material; and

G. examining said bound material for the presence of said labeled material.

25. The method of Claim 24, wherein said receptor recognition factor has the following characteristics:

a) It interacts with an interferon-γ-bound receptor kinase complex;

b) It is a tyrosine kinase substrate; and

c) When phosphorylated, it serves as a DNA binding protein.

26. The method of Claim 25, wherein said receptor recognition factor is further characterized in that:

d) Interferon-γ-dependent activation of said factor occurs without new protein synthesis; and

e) Activation of said factor appears within minutes of interferon-γ treatment, achieves maximum extent between 15 and 30 minutes thereafter, and then disappears after 2-3 hours.

27. The method of Claim 24 comprising a method for measuring the presence and activity of a polypeptide ligand associated with a given invasive stimulus in mammals.

28. The method of Claim 27 wherein said invasive stimulus is an infection.

29. The method of Claim 28 wherein said invasive stimulus is selected from the group consisting of viral infection, protozoan infection, tumorous mammalian cells, and toxins.

30. The method of Claim 24 comprising a method for determining the presence of invasive or idiopathic stimuli in mammals.

31. A method for measuring the binding sites for a receptor recognition factor, said receptor recognition factor having the following characteristics:

apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene;

an activity demonstrably unaffected by the presence or concentration of second messengers;

direct interaction with tyrosine kinase domains; and

a perceived absence of interaction with G-proteins; wherein the binding sites for said receptor recognition factor are measured by:

A. providing at least one sample of said receptor recognition factor;

B. placing a detectible label on said receptor recognition factor sample;

C. placing the labeled receptor recognition factor sample in contact with a biological sample from a mammal in which binding sites for said receptor recognition factor are suspected;

D. examining said biological sample in binding studies for the presence of said labeled receptor recognition factor.

32. A method of testing the ability of a drug or other entity to modulate the activity of a receptor recognition factor which comprises culturing a colony of test cells which has a receptor for the receptor recognition factor in a growth medium containing the receptor recognition factor, adding the drug under test and thereafter measuring the reactivity of said receptor recognition factor with the receptor on said colony of test cells, said receptor recognition factor having the following characteristics:

a) apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene;

b) an activity demonstrably unaffected by the presence or concentration of second messengers;

c) direct interaction with tyrosine kinase domains; and

d) a perceived absence of interaction with G-proteins.

33. The method of either of Claims 31 or 32, wherein said receptor recognition factor has the following characteristics:

a) It interacts with an interferon-γ-bound receptor kinase complex;

b) It is a tyrosine kinase substrate; and

c) When phosphorylated, it serves as a DNA binding protein.

34. The method of Claim 33, wherein said receptor recognition factor is further characterized in that:

d) Interferon-γ-dependent activation of said factor occurs without new protein synthesis; and

e) Activation of said factor appears within minutes of interferon-γ treatment, achieves maximum extent between 15 and 30 minutes thereafter, and then disappears after 2-3 hours.

35. An assay system for screening drugs and other agents for ability to modulate the production of a receptor recognition factor, comprising an observable cellular test colony inoculated with a drug or agent, and yielding a resulting supernatant, said supernatant then to be examined for the presence of said receptor recognition factor, said receptor recognition factor having the following characteristics:

a) apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene;

b) an activity demonstrably unaffected by the presence or concentration of second messengers;

c) direct interaction with tyrosine kinase domains; and

d) a perceived absence of interaction with G-proteins.

36. The assay system of Claim 35, wherein said receptor recognition factor has the following characteristics:

a) It interacts with an interferon-γ-bound receptor kinase complex;

b) It is a tyrosine kinase substrate; and

c) When phosphorylated, it serves as a DNA binding protein.

37. The assay system of Claim 36, wherein said receptor recognition factor is further characterized in that:

d) Interferon-γ-dependent activation of said factor occurs without new protein synthesis; and

e) Activation of said factor appears within minutes of interferon-γ treatment, achieves maximum extent between 15 and 30 minutes thereafter, and then disappears after 2-3 hours.

38. A test kit for the demonstration of a receptor recognition factor in a eukaryotic cellular sample, comprising:

A. a predetermined amount of at least one labeled immunochemically reactive component obtained by the direct or indirect attachment of said receptor recognition factor or a specific binding partner thereto, to a detectable label, said receptor recognition factor having the following characteristics: apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene; an activity demonstrably unaffected by the presence or concentration of second messengers; direct interaction with tyrosine kinase domains; and a perceived absence of interaction with G-proteins;

B. other reagents; and

C. directions for use of said kit.

39. The test kit of Claim 38, wherein said receptor recognition factor has the following characteristics:

a) It interacts with an interferon-γ-bound receptor kinase complex;

b) It is a tyrosine kinase substrate; and

c) When phosphorylated, it serves as a DNA binding protein.

40. The test kit of Claim 39, wherein said receptor recognition factor is further characterized in that:

d) Interferon-γ-dependent activation of said factor occurs without new protein synthesis; and

e) Activation of said factor appears within minutes of interferon-γ treatment, achieves maximum extent between 15 and 30 minutes thereafter, and then disappears after 2-3 hours.

41. The test kit of any of Claims 38-40, wherein said labeled immunochemically reactive component is selected from the group consisting of polyclonal antibodies to the receptor recognition factor, monoclonal antibodies to the receptor recognition factor, fragments thereof, and mixtures thereof.

42. A method of preventing and/or treating cellular debilitations, derangements and/or dysfunctions and/or other disease states in mammals, comprising administering to a mammal a therapeutically effective amount of a material selected from the group consisting of a receptor recognition factor, an agent capable of promoting the production and/or activity of said receptor recognition factor, an agent capable of mimicking the activity of said receptor recognition factor, an agent capable of inhibiting the production and/or activity of said receptor recognition factor, and mixtures thereof, or a specific binding partner thereto, said receptor recognition factor having the following characteristics:

a) apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene;

b) an activity demonstrably unaffected by the presence or concentration of second messengers;

c) direct interaction with tyrosine kinase domains; and

d) a perceived absence of interaction with G-proteins.

43. The method of Claim 42, wherein said receptor recognition factor has the following characteristics:

a) It interacts with an interferon-γ-bound receptor kinase complex;

b) It is a tyrosine kinase substrate; and

c) When phosphorylated, it serves as a DNA binding protein.

44. The method of Claim 43, wherein said receptor recognition factor is further characterized in that:

d) Interferon-γ-dependent activation of said factor occurs without new protein synthesis; and

e) Activation of said factor appears within minutes of interferon-γ treatment, achieves maximum extent between 15 and 30 minutes thereafter, and then disappears after 2-3 hours.

45. The method of Claim 42 wherein said disease states include chronic viral hepatitis, hairy cell leukemia, and tumorous conditions.

46. The method of Claim 42 wherein said receptor recognition factor is administered to modulate the course of therapy where interferon is being administered as the primary therapeutic agent.

47. The method of Claim 42 wherein said agent is administered to modulate the course of therapy where interferon is being administered as the primary therapeutic agent.

48. The method of Claim 42 wherein said receptor recognition factor is administered to modulate the course of therapy where interferon is being co-administered with one or more additional therapeutic agents.

49. The method of Claim 42 wherein said agent is administered to modulate the course of therapy where interferon is being co-administered with one or more additional therapeutic agents.

50. A pharmaceutical composition for the treatment of cellular debilitation, derangement and/or dysfunction in mammals, comprising:

A. a therapeutically effective amount of a material selected from the group consisting of a receptor recognition factor, an agent capable of promoting the production and/or activity of said receptor recognition factor, an agent capable of mimicking the activity of said receptor recognition factor, an agent capable of inhibiting the production and/or activity of said receptor recognition factor, and mixtures thereof, or a specific binding partner thereto, said receptor recognition factor having the following characteristics: apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene; an activity demonstrably unaffected by the presence or concentration of second messengers; direct interaction with tyrosine kinase domains; and a perceived absence of interaction with G-proteins; and

B. a pharmaceutically acceptable carrier.

51. The pharmaceutical composition of Claim 50, wherein said receptor recognition factor has the following characteristics:

a) It interacts with an interferon-γ-bound receptor kinase complex;

b) It is a tyrosine kinase substrate; and

c) When phosphorylated, it serves as a DNA binding protein.

52. The pharmaceutical composition of Claim 51, wherein said receptor recognition factor is further characterized in that:

d) Interferon-γ-dependent activation of said factor occurs without new protein synthesis; and

e) Activation of said factor appears within minutes of interferon-γ treatment, achieves maximum extent between 15 and 30 minutes thereafter, and then disappears after 2-3 hours.

53. A receptor recognition factor implicated in the transcriptional stimulation of genes in target cells in response to the binding of a specific polypeptide ligand to its cellular receptor on said target cell, said receptor recognition factor having the following properties:

a) it is present in cytoplasm;

b) it undergoes tyrosine phosphorylation upon treatment of cells with IFNα;

c) it activates transcription of an interferon stimulated gene;

d) it stimulates either an ISRE-dependent or a gamma activated site (GAS)-dependent transcription *in vivo*;

e) it interacts with IFNα cellular receptors, and

f) it undergoes nuclear translocation upon stimulation of the IFN cellular receptors with IFNα.

54. A receptor recognition factor implicated in the transcriptional stimulation of genes in target cells in response to the binding of an interferon or interferon-related polypeptide ligand to its cellular receptor on said target cell, said receptor recognition factor having the following properties:

a) it is present *in vivo* in mammalian cytoplasm before activation of cellular IFN receptors;

b) it contains tyrosine sites that are phosphorylated in response to IFN stimulation of IFN receptors;

c) it has a molecular weight selected from the group consisting of 48 kD, 84 kD, 91 kD and 113 kD, and

d) when phosphorylated, it recognizes an ISRE in the cell nucleus.

55. The receptor recognition factor of either of Claims 53 or 54 in phosphorylated form.

56. An antibody which recognizes a phosphorylated ISGF3 polypeptide or a fragment thereof in phosphorylated form.

57. An antibody produced by injecting a substantially immunocompetent host with an antibody-producing effective amount of an ISGF3 polypeptide, and harvesting said antibody, said ISGF3 polypeptide having the following properties:

a) it has a molecular weight of about 48 kD, 84 kD, 91 kD or 113 kD;

b) it can be isolated from mammalian cytoplasm;

c) it contains tyrosine residues that are subject to phosphorylation *in vivo* upon treatment of cells with IFNα;

d) it can activate transcription of an interferon stimulated gene *in vivo*;

e) it can stimulate ISRE-dependent transcription *in vivo*;

f) it can interact with IFNα cellular receptors, and

g) it can undergo nuclear translocation upon stimulation of IFN cellular receptors with IFNα.

58. The antibody of either of Claims 56 or 57 which is monoclonal.

59. The antibody of either of Claims 56 or 57 which is polyclonal.

60. A recombinant virus transformed with the DNA molecule, or a derivative or fragment thereof, in accordance with Claim 17.

61. A recombinant virus transformed with the DNA molecule, or a derivative or fragment thereof, in accordance with Claim 18.

62. A method of enhancing the activity of IFNα and/or IFNγ in a mammal in need of such treatment, comprising administering to said mammal an effective

amount of a compound which (a) enhances the phosphorylation of the receptor recognition factor of Claim 1, or (b) inhibits the activity of a phosphatase enzyme which would otherwise reduce the level of phosphorylated receptor recognition factor.

63. A method of treating (a) chronic viral hepatitis or (b) hairy cell leukemia, in a mammal in need of such treatment, comprising administering to said mammal an effective amount of a compound which (a) enhances the phosphorylation of the receptor recognition factor of Claim 1, or (b) decreases the level of phosphate removal from phosphorylated receptor recognition factor.

64. The method of Claim 62 wherein the activity of exogenous IFNα and/or IFNγ is enhanced.

65. The method of Claim 62 wherein the activity of endogenous IFNα and/or IFNγ is enhanced.

66. The method of Claim 64 wherein the compound and IFNα and/or IFNγ are administered concurrently to the mammal in need of such treatment.

67. A method of determining the interferon-related pharmacological activity of a compound comprising:

administering the compound to a mammal;

determining the level of phosphorylated receptor recognition factor present; and

comparing the level of receptor recognition factor-phosphate to a standard.

68. In a method of treating hepatitis or leukemia in a mammal, wherein IFNγ is administered in an amount effective for treating such hepatitis or leukemia, the improvement comprising administering to said mammal a receptor recognition

factor of Claim 1 or a derivative thereof in an amount effective for enhancing the activity of said IFNγ.

69. A method of determining the interferon-related pharmacological activity of a compound comprising:

administering the compound to a mammal;

determining the level of phosphorylated ISGF3 proteins present; and

comparing the level of ISGF3 protein-phosphate to a standard.

70. In a method of treating hepatitis or leukemia in a mammal, wherein IFNα is administered in an amount effective for treating such hepatitis or leukemia, the improvement comprising administering to said mammal an ISGF3 protein or a derivative thereof in an amount effective for enhancing the activity of said IFNα.

71. The method of Claim 70 wherein a derivative of said ISGF3 protein is administered.

72. The method of Claim 71, wherein said ISGF3 protein has a molecular weight of about 48 kD, 84 kD, 91 kD or 113 kD.

73. The method of Claim 71 wherein the derivative is a phosphorylated ISGF3 protein.

74. The recombinant DNA molecule of Claim 18 comprising plasmid pGEX-3X, clone E3 or plasmid pGEX-3X, clone E4.

75. An antisense nucleic acid against a receptor recognition factor mRNA comprising a nucleic acid sequence hybridizing to said mRNA.

76. The antisense nucleic acid of Claim 75 comprising RNA.

77. The antisense nucleic acid of Claim 75 comprising DNA.

78. The antisense nucleic acid of Claim 75 which binds to the initiation codon of any of said mRNAs.

79. A recombinant DNA molecule having a DNA sequence which, on transcription, produces an antisense ribonucleic acid against a receptor recognition factor mRNA, said antisense ribonucleic acid comprising an nucleic acid sequence hybridizing to said mRNA.

80. A receptor recognition factor-producing cell line transfected with a recombinant DNA molecule having a DNA sequence which, on transcription, produces an antisense ribonucleic acid against a receptor recognition factor mRNA, said antisense ribonucleic acid comprising an nucleic acid sequence hybridizing to said mRNA.

81. A method for creating a cell line which exhibits reduced expression of a receptor recognition factor, comprising transfecting a recognition factor-producing cell line with a recombinant DNA molecule having a DNA sequence which, on transcription, produces an antisense ribonucleic acid against a receptor recognition factor mRNA, said antisense ribonucleic acid comprising an nucleic acid sequence hybridizing to said mRNA.

82. A ribozyme that cleaves receptor recognition factor mRNA.

83. The ribozyme of Claim 82 further comprising a *Tetrahymena*-type ribozyme.

84. The ribozyme of Claim 82 further comprising a Hammerhead-type ribozyme.

85. A recombinant DNA molecule having a DNA sequence which, upon transcription, produces a ribozyme that cleaves receptor recognition factor mRNA.

86. A receptor recognition factor-producing cell line transfected with a recombinant DNA molecule having a DNA sequence which, upon transcription, produces a ribozyme that cleaves receptor recognition factor mRNA.

87. A method for creating a cell line which exhibits reduced expression of a receptor recognition factor, comprising transfecting a recognition factor-producing cell line with a recombinant DNA molecule that produces on transcription a ribozyme that cleaves receptor recognition factor mRNA.

Fig 1a

```
                                    1
                                    met ala gln trp glu met leu gln
ACTGCAACCCTAATCAGAGCCCAA            ATG GCG CAG TGG GAA ATG CTG CAG

    10                                                  20
asn leu asp ser pro phe gln asp gln leu his gln leu tyr ser
AAT CTT GAC AGC CCC TTT CAG GAT CAG CTG CAC CAG CTT TAC TCG

                                30
his ser leu leu pro val asp ile arg gln tyr leu ala val trp
CAC AGC CTC CTG CCT GTG GAC ATT CGA CAG TAC TTG GCT GTC TGG

    40                                                  50
ile glu asp gln asn trp gln glu ala ala leu gly ser asp asp
ATT GAA GAC CAG AAC TGG CAG GAA GCT GCA CTT GGG AGT GAT GAT

                                60
ser lys ala thr met leu phe phe his phe leu asp gln leu asn
TCC AAG GCT ACC ATG CTA TTC TTC CAC TTC TTG GAT CAG CTG AAC

    70                                                  80
tyr glu cys gly arg cys ser gln asp pro glu ser leu leu leu
TAT GAG TGT GGC CGT TGC AGC CAG GAC CCA GAG TCC TTG TTG CTG

                                90
gln his asn leu arg lys phe cys arg asp ile gln pro phe ser
CAG CAC AAT TTG CGG AAA TTC TGC CGG GAC ATT CAG CCC TTT TCC

    100                                                 110
gln asp pro thr gln leu ala glu met ile phe asn leu leu leu
CAG GAT CCT ACC CAG TTG GCT GAG ATG ATC TTT AAC CTC CTT CTG

                               120
glu glu lys arg ile leu ile gln ala gln arg ala gln leu glu
GAA GAA AAA AGA ATT TTG ATC CAG GCT CAG AGG GCC CAA TTG GAA

    130                                                 140
gln gly glu pro val leu glu thr pro val glu ser gln gln his
CAA GGA GAG CCA GTT CTC GAA ACA CCT GTG GAG AGC CAG CAA CAT

                               150
glu ile glu ser arg ile leu asp leu arg ala met met glu lys
GAG ATT GAA TCC CGG ATC CTG GAT TTA AGG GCT ATG ATG GAG AAG

    160                                                 170
leu val lys ser ile ser gln leu lys asp gln gln asp val phe
CTG GTA AAA TCC ATC AGC CAA CTG AAA GAC CAG CAG GAT GTC TTC

                               180
```

FIG 1b

Session Name: rb

```
cys phe|arg tyr lys ile gln ala lys gly lys thr pro ser leu
TGC TTC CGA TAT AAG ATC CAG GCC AAA GGG AAG ACA CCC TCT CTG

                                                    200
    190
asp |pro his gln thr lys glu gln lys ile leu gln glu thr leu
GAC |CCC CAT CAG ACC AAA GAG CAG AAG ATT CTG CAG GAA ACT CTC

                            210
asn glu leu asp lys arg arg lys glu val leu asp ala ser lys
AAT GAA CTG GAC AAA AGG AGA AAG GAG GTG CTG GAT GCC TCC AAA

    220                                             230
ala leu leu gly arg leu thr thr leu ile glu leu leu leu pro
GCA CTG CTA GGC CGA TTA ACT ACC CTA ATC GAG CTA CTG CTG CCA

                            240
lys leu glu glu trp lys ala gln gln gln lys ala cys ile arg
AAG TTG GAG GAG TGG AAG GCC CAG CAG CAA AAA GCC|TGC ATC AGA

    250                                             260
ala pro ile asp his gly leu glu gln leu glu |thr trp phe thr
GCT CCC ATT GAC CAC GGG TTG GAA CAG CTG GAG |ACA TGG TTC ACA

                            270
ala gly ala lys leu leu phe his leu arg gln leu leu lys glu
GCT GGA GCA AAG CTG TTG TTT CAC CTG AGG CAG CTG CTG AAG GAG

    280                                             290
leu lys gly leu ser cys leu val ser tyr gln asp asp pro leu
CTG AAG GGA CTG AGT TGC CTG GTT AGC|TAT CAG GAT GAC CCT CTG

                            300
thr lys gly val asp leu arg asn |ala gln val thr glu leu leu
ACC AAA GGG GTG GAC CTA CGC AAC GCC CAG GTC ACA GAG TTG CTA

    310                                             320
gln arg leu leu his arg ala |phe val val glu thr gln pro cys
CAG CGT CTG CTC CAC AGA GCC|TTT GTG GTA GAA ACC CAG CCC TGC

                            330
met pro gln thr pro his arg pro leu ile leu lys thr gly ser
ATG CCC CAA ACT CCC CAT CGA CCC CTC ATC CTC AAG ACT GGC AGC

    340                                             350
lys phe thr val arg|thr arg leu leu val arg leu gln glu gly
AAG TTC ACC GTC CGA|ACA AGG CTG CTG GTG AGA CTC CAG GAA GGC

                            360
asn glu ser leu thr val glu val ser ile asp arg asn pro pro
AAT GAG TCA CTG ACT GTG GAA GTC TCC ATT GAC AGG AAT CCT CCT

    370                                             380
gln leu gln gly phe arg lys phe asn ile leu thr ser asn gln
CAA TTA CAA GGC TTC CGG AAG TTC AAC ATT CTG ACT TCA AAC CAG

                            390
lys thr leu thr pro glu lys gly gln ser gln gly leu ile trp
```

Fig 1c

Session Name: rb

```
AAA ACT TTG ACC CCC GAG AAG GGG CAG AGT CAG GGT TTG ATT TGG

                                                        410
    400
asp phe gly tyr leu thr leu val glu gln arg ser gly gly ser
GAC TTT GGT TAC CTG ACT CTG GTG GAG CAA CGT TCA GGT GGT TCA

                            420
gly lys gly ser asn lys gly pro leu gly val thr glu glu leu
GGA AAG GGC AGC AAT AAG GGG CCA CTA GGT GTG ACA GAG GAA CTG

    430                                                 440
his ile ile ser phe thr val lys tyr thr tyr gln gly leu lys
CAC ATC ATC AGC TTC ACG GTC AAA TAT ACC TAC CAG GGT CTG AAG

                            450
gln glu leu lys thr asp thr leu pro val val ile ile ser asn
CAG GAG CTG AAA ACG GAC ACC CTC CCT GTG GTG ATT ATT TCC AAC

    460                                                 470
met asn gln leu ser ile ala trp ala ser val leu trp phe asn
ATG AAC CAG CTC TCA ATT GCC TGG GCT TCA GTT CTC TGG TTC AAT

                            480
leu leu ser pro asn leu gln asn gln gln phe phe ser asn pro
TTG CTC AGC CCA AAC CTT CAG AAC CAG CAG TTC TTC TCC AAC CCC

    490                                                 500
pro lys ala pro trp ser leu leu gly pro ala leu ser trp gln
CCC AAG GCC CCC TGG AGC TTG CTG GGC CCT GCT CTC AGT TGG CAG

                            510
phe ser ser tyr val gly arg gly leu asn ser asp gln leu ser
TTC TCC TCC TAT GTT GGC CGA GGC CTC AAC TCA GAC CAG CTG AGC

    520                                                 530
met leu arg asn lys leu phe gly gln asn cys arg thr glu asp
ATG CTG AGA AAC AAG CTG TTC GGG CAG AAC TGT AGG ACT GAG GAT

                            540
pro leu leu ser trp ala asp phe thr lys arg glu ser pro pro
CCA TTA TTG TCC TGG GCT GAC TTC ACT AAG CGA GAG AGC CCT CCT

    550                                                 560
gly lys leu pro phe trp thr trp leu asp lys ile leu glu leu
GGC AAG TTA CCA TTC TGG ACA TGG CTG GAC AAA ATT CTG GAG TTG

                            570
val his asp his leu lys asp leu trp asn asp gly arg ile met
GTA CAT GAC CAC CTG AAG GAT CTC TGG AAT GAT GGA CGC ATC ATG

    580                                                 590
gly phe val ser arg ser gln glu arg arg leu leu lys lys thr
GGC TTT GTG AGT CGG AGC CAG GAG CGC CGG CTG CTG AAG AAG ACC

                            600
met ser gly thr phe leu leu arg phe ser glu ser ser glu gly
ATG TCT GGC ACC TTT CTA CTG CGC TTC AGT GAA TCG TCA GAA GGG
```

FIG 1d

Session Name: rb

```
    610                                                 620
gly ile thr cys ser trp val glu his gln asp asp asp lys val
GGC ATT ACC TGC TCC TGG GTG GAG CAC CAG GAT GAT GAC AAG GTG

                        630
leu ile tyr ser val gln pro tyr thr lys glu val leu gln ser
CTC ATC TAC TCT GTG CAA CCG TAC ACG AAG GAG GTG CTG CAG TCA

    640                                                 650
leu pro leu thr glu ile ile arg his tyr gln leu leu thr glu
CTC CCG CTG ACT GAA ATC ATC CGC CAT TAC CAG TTG CTC ACT GAG

                        660
glu asn ile pro glu asn pro leu arg phe leu tyr pro arg ile
GAG AAT ATA CCT GAA AAC CCA CTG CGC TTC CTC TAT CCC CGA ATC

    670                                                 680
pro arg asp glu ala phe gly cys tyr tyr gln glu lys val asn
CCC CGG GAT GAA GCT TTT GGG TGC TAC TAC CAG GAG AAA GTT AAT

                        690
leu gln glu arg arg lys tyr leu lys his arg leu ile val val
CTC CAG GAA CGG AGG AAA TAC CTG AAA CAC AGG CTC ATT GTG GTC

    700                                                 710
ser asn arg gln val asp glu leu gln gln pro leu glu leu lys
TCT AAT AGA CAG GTG GAT GAA CTG CAA CAA CCG CTG GAG CTT AAG

                        720
pro glu pro glu leu glu ser leu glu leu glu leu gly leu val
CCA GAG CCA GAG CTG GAG TCA TTA GAG CTG GAA CTA GGG CTG GTG

    730                                                 740
pro glu pro glu leu ser leu asp leu glu pro leu leu lys ala
CCA GAG CCA GAG CTC AGC CTG GAC TTA GAG CCA CTG CTG AAG GCA

                        750
gly leu asp leu gly pro glu leu glu ser val leu glu ser thr
GGG CTG GAT CTG GGG CCA GAG CTA GAG TCT GTG CTG GAG TCC ACT

    760                                                 770
leu glu pro val ile glu pro thr leu cys met val ser gln thr
CTG GAG CCT GTG ATA GAG CCC ACA CTA TGC ATG GTA TCA CAA ACA

                        780
val pro glu pro asp gln gly pro val ser gln pro val pro glu
GTG CCA GAG CCA GAC CAA GGA CCT GTA TCA CAG CCA GTG CCA GAG

    790                                                 800
pro asp leu pro cys asp leu arg his leu asn thr glu pro met
CCA GAT TTG CCC TGT GAT CTG AGA CAT TTG AAC ACT GAG CCA ATG

                        810
glu ile phe arg asn cys val lys ile glu glu ile met pro asn
GAA ATC TTC AGA AAC TGT GTA AAG ATT GAA GAA ATC ATG CCG AAT
```

Fig 1e

Session Name: rb

```
    820                                                 830
gly asp pro leu leu ala gly gln asn thr val asp glu val tyr
GGT GAC CCA CTG TTG GCT GGC CAG AAC ACC GTG GAT GAG GTT TAC

                        840
val ser arg pro ser his phe tyr thr asp gly pro leu met pro
GTC TCC CGC CCC AGC CAC TTC TAC ACT GAT GGA CCC TTG ATG CCT

    850 851
ser asp phe AM
TCT GAC TTC TAG GAACCACATTTCCTCTGTTCTTTTCATATCTCTTTGCCCTTCCTA

CTCCTCATAGCATGATATTGTTCTCCAAGGATGGGAATCAGGCATGTGTCCCTTCCAAGC

TGTGTTAACTGTTCAAACTCAGGCCTGTGTGACTCCATTGGGGTGAGAGGTGAAAGCATA

ACATGGGTACAGAGGGGACAACAATGAATCAGAACAGATGCTGAGCCATAGGTCTAAATA

GGATCCTGGAGGCTGCCTGCTGTGCTGGGAGGTATAGGGGTCCTGGGGGCAGGCCAGGGC

AGTTGACAGGTACTTGGAGGGCTCAGGGCAGTGGCTTCTTTCCAGTATGGAAGGATTTCA

ACATTTTAATAGTTGGTTAGGCTAAACTGGTGCATACTGGCATTGGCCTTGGTGGGGAGC

ACAGACACAGGATAGGACTCCATTTCTTTCTTCCATTCCTTCATGTCTAGGATAACTTGC

TTTCTTCTTTCCTTTACTCCTGGCTCAAGCCCTGAATTTCTTCTTTTCCTGCAGGGGTTG

AGAGCTTTCTGCCTTAGCCTACCATGTGAAACTCTACCCTGAAGAAAGGGATGGATAGGA

AGTAGACCTCTTTTTCTTACCAGTCTCCTCCCCTACTCTGCCCCCTAAGCTGGCTGTACC

TGTTCCTCCCCCATAAAATGATCCTGCCAATCTAAAAAAAAAA
```

FIG 2a

```
GCCGAGCCCCTCCGCAGACTCTGCGCCGGAAAGTTTCATTTGCTGTATGCCATCCTCGA

GAGCTGTCTAGGTTAACGTTCGCACTCTGTGTATATAACCTCGACAGTCTTGGCACCTA

ACGTGCTGTGCGTAGCTGCTCCTTTGGTTGAATCCCCAGGCCCTTGTTGGGGCACAAGG


          1                                                  10
        met ser gln trp tyr glu leu gln gln leu asp ser lys
TGGCAGG ATG TCT CAG TGG TAC GAA CTT CAG CAG CTT GAC TCA AAA

                          20
phe leu glu gln val his gln leu tyr asp asp ser phe pro met
TTC CTG GAG CAG GTT CAC CAG CTT TAT GAT GAC AGT TTT CCC ATG

     30                                                  40
glu ile arg gln tyr leu ala gln trp leu glu lys gln asp trp
GAA ATC AGA CAG TAC CTG GCA CAG TGG TTA GAA AAG CAA GAC TGG

                          50
glu his ala ala asn asp val ser phe ala thr ile arg phe his
GAG CAC GCT GCC AAT GAT GTT TCA TTT GCC ACC ATC CGT TTT CAT

     60                                                  70
asp leu leu ser gln leu asp asp gln tyr ser arg phe ser leu
GAC CTC CTG TCA CAG CTG GAT GAT CAA TAT AGT CGC TTT TCT TTG

                          80
glu asn asn phe leu leu gln his asn ile arg lys ser lys arg
GAG AAT AAC TTC TTG CTA CAG CAT AAC ATA AGG AAA AGC AAG CGT

     90                                                 100
asn leu gln asp asn phe gln glu asp pro ile gln met ser met
AAT CTT CAG GAT AAT TTT CAG GAA GAC CCA ATC CAG ATG TCT ATG

                         110
ile ile tyr ser cys leu lys glu glu arg lys ile leu glu asn
ATC ATT TAC AGC TGT CTG AAG GAA GAA AGG AAA ATT CTG GAA AAC

    120                                                 130
ala gln arg phe asn gln ala gln ser gly asn ile gln ser thr
GCC CAG AGA TTT AAT CAG GCT CAG TCG GGG AAT ATT CAG AGC ACA

                         140
val met leu asp lys gln lys glu leu asp ser lys val arg asn
GTG ATG TTA GAC AAA CAG AAA GAG CTT GAC AGT AAA GTC AGA AAT

    150                                                 160
val lys asp lys val met cys ile glu his glu ile lys ser leu
GTG AAG GAC AAG GTT ATG TGT ATA GAG CAT GAA ATC AAG AGC CTG

                         170
glu asp leu gln asp glu tyr asp phe lys cys lys thr leu gln
GAA GAT TTA CAA GAT GAA TAT GAC TTC AAA TGC AAA ACC TTG CAG
```

FIG 2b

Session Name: rb

```
    180                                                 190
asn arg glu his glu thr asn gly val ala lys ser asp gln lys
AAC AGA GAA CAC GAG ACC AAT GGT GTG GCA AAG AGT GAT CAG AAA

                        200
gln glu gln leu leu leu lys lys met tyr leu met leu asp asn
CAA GAA CAG CTG TTA CTC AAG AAG ATG TAT TTA ATG CTT GAC AAT

    210                                                 220
lys arg lys glu val val his lys ile ile glu leu leu asn val
AAG AGA AAG GAA GTA GTT CAC AAA ATA ATA GAG TTG CTG AAT GTC

                        230
thr glu leu thr gln asn ala leu ile asn asp glu leu val glu
ACT GAA CTT ACC CAG AAT GCC CTG ATT AAT GAT GAA CTA GTG GAG

    240                                                 250
trp lys arg arg gln gln ser ala cys ile gly gly pro pro asn
TGG AAG CGG AGA CAG CAG AGC GCC TGT ATT GGG GGG CCG CCC AAT

                        260
ala cys leu asp gln leu gln gln val arg gln gln leu lys lys
GCT TGC TTG GAT CAG CTG CAG CAA GTT CGG CAG CAG CTT AAA AAG

    270                                                 280
leu glu glu leu glu gln lys tyr thr tyr glu his asp pro ile
TTG GAG GAA TTG GAA CAG AAA TAC ACC TAC GAA CAT GAC CCT ATC

                        290
thr lys asn lys gln val leu trp asp arg thr phe ser leu phe
ACA AAA AAC AAA CAA GTG TTA TGG GAC CGC ACC TTC AGT CTT TTC

    300                                                 310
gln gln leu ile gln ser ser phe val val glu arg gln pro cys
CAG CAG CTC ATT CAG AGC TCG TTT GTG GTG GAA AGA CAG CCG TGC

                        320
met pro thr his pro gln arg pro leu val leu lys thr gly val
ATG CCA ACG CAC CCT CAG AGG CCG CTG GTC TTG AAG ACA GGG GTC

    330                                                 340
gln phe thr val lys leu arg leu leu val lys leu gln glu leu
CAG TTC ACT GTG AAG TTG AGA CTG TTG GTG AAA TTG CAA GAG CTG

                        350
asn tyr asn leu lys val lys val leu phe asp lys asp val asn
AAT TAT AAT TTG AAA GTC AAA GTC TTA TTT GAT AAA GAT GTG AAT

    360                                                 370
glu arg asn thr val lys gly phe arg lys phe asn ile leu gly
GAG AGA AAT ACA GTA AAA GGA TTT AGG AAG TTC AAC ATT TTG GGC

                        380
thr his thr lys val met asn met glu glu ser thr asn gly ser
ACG CAC ACA AAA GTG ATG AAC ATG GAG GAG TCC ACC AAT GGC AGT
```

FIG 2C

Session Name: rb

```
    390                                                 400
leu ala ala glu phe arg his leu gln leu lys glu gln lys asn
CTG GCG GCT GAA TTT CGG CAC CTG CAA TTG AAA GAA CAG AAA AAT

                        410
ala gly thr arg thr asn glu gly pro leu ile val thr glu glu
GCT GGC ACC AGA ACG AAT GAG GGT CCT CTC ATC GTT ACT GAA GAG

    420                                                 430
leu his ser leu ser phe glu thr gln leu cys gln pro gly leu
CTT CAC TCC CTT AGT TTT GAA ACC CAA TTG TGC CAG CCT GGT TTG

                        440
val ile asp leu glu thr thr ser leu pro val val val ile ser
GTA ATT GAC CTC GAG ACG ACC TCT CTG CCC GTT GTG GTG ATC TCC

    450                                                 460
asn val ser gln leu pro ser gly trp ala ser ile leu trp tyr
AAC GTC AGC CAG CTC CCG AGC GGT TGG GCC TCC ATC CTT TGG TAC

                        470
asn met leu val ala glu pro arg asn leu ser phe phe leu thr
AAC ATG CTG GTG GCG GAA CCC AGG AAT CTG TCC TTC TTC CTG ACT

    480                                                 490
pro pro cys ala arg trp ala gln leu ser glu val leu ser trp
CCA CCA TGT GCA CGA TGG GCT CAG CTT TCA GAA GTG CTG AGT TGG

                        500
gln phe ser ser val thr lys arg gly leu asn val asp gln leu
CAG TTT TCT TCT GTC ACC AAA AGA GGT CTC AAT GTG GAC CAG CTG

    510                                                 520
asn met leu gly glu lys leu leu gly pro asn ala ser pro asp
AAC ATG TTG GGA GAG AAG CTT CTT GGT CCT AAC GCC AGC CCC GAT

                        530
gly leu ile pro trp thr arg phe cys lys glu asn ile asn asp
GGT CTC ATT CCG TGG ACG AGG TTT TGT AAG GAA AAT ATA AAT GAT

    540                                                 550
lys asn phe pro phe trp leu trp ile glu ser ile leu glu leu
AAA AAT TTT CCC TTC TGG CTT TGG ATT GAA AGC ATC CTA GAA CTC

                        560
ile lys lys his leu leu pro leu trp asn asp gly cys ile met
ATT AAA AAA CAC CTG CTC CCT CTC TGG AAT GAT GGG TGC ATC ATG

    570                                                 580
gly phe ile ser lys glu arg glu arg ala leu leu lys asp gln
GGC TTC ATC AGC AAG GAG CGA GAG CGT GCC CTG TTG AAG GAC CAG

                        590
gln pro gly thr phe leu leu arg phe ser glu ser ser arg glu
CAG CCG GGG ACC TTC CTG CTG CGG TTC AGT GAG AGC TCC CGG GAA

    600                                                 610
```

Fig 2d

Session Name: rb

```
gly ala ile thr phe thr trp val glu arg ser gln asn gly gly
GGG GCC ATC ACA TTC ACA TGG GTG GAG CGG TCC CAG AAC GGA GGC

                          620
glu pro asp phe his ala val glu pro tyr thr lys lys glu leu
GAA CCT GAC TTC CAT GCG GTT GAA CCC TAC ACG AAG AAA GAA CTT

    630                                               640
ser ala val thr phe pro asp ile ile arg asn tyr lys val met
TCT GCT GTT ACT TTC CCT GAC ATC ATT CGC AAT TAC AAA GTC ATG

                          650
ala ala glu asn ile pro glu asn pro leu lys tyr leu tyr pro
GCT GCT GAG AAT ATT CCT GAG AAT CCC CTG AAG TAT CTG TAT CCA

    660                                               670
asn ile asp lys asp his ala phe gly lys tyr tyr ser arg pro
AAT ATT GAC AAA GAC CAT GCC TTT GGA AAG TAT TAC TCC AGG CCA

                          680
lys glu ala pro glu pro met glu leu asp gly pro lys gly thr
AAG GAA GCA CCA GAG CCA ATG GAA CTT GAT GGC CCT AAA GGA ACT

    690                                               700
gly tyr ile lys thr glu leu ile ser val ser glu val his pro
GGA TAT ATC AAG ACT GAG TTG ATT TCT GTG TCT GAA GTT CAC CCT

                          710
ser arg leu gln thr thr asp asn leu leu pro met ser pro glu
TCT AGA CTT CAG ACC ACA GAC AAC CTG CTC CCC ATG TCT CCT GAG

    720                                               730
glu phe asp glu val ser arg ile val gly ser val glu phe asp
GAG TTT GAC GAG GTG TCT CGG ATA GTG GGC TCT GTA GAA TTC GAC

                      739
ser met met asn thr val AM
AGT ATG ATG AAC ACA GTA TAG AGCATGAATTTTTTCATCTTCTCTGGCGACAG

TTTTCCTTCTCATCTGTGATTCCCTCCTGCTACTCTGTTCCTTCACATCCTGTGTTTCTA

GGGAAATGAAAGAAAGGCCAGCAAATTCGCTGCAACCTGTTGATAGCAAGTGAATTTTTC

TCTAACTCAGAAACATCAGTTACTCTGAAGGGCATCATGCATCTTACTGAAGGTAAAATT

GAAAGGCATTCTCTGAAGAGTGGGTTTCACAAGTGAAAAACATCCAGATACACCCAAAGT

ATCAGGACGAGAATGAGGGTCCTTTGGGAAAGGAGAAGTTAAGCAACATCTAGCAAATGT

TATGCATAAAGTCAGTGCCCAACTGTTATAGGTTGTTGGATAAATCAGTGGTTATTTAGG

GAACTGCTTGACGTAGGAACGGTAAATTTCTGTGGGAGAATTCTTACATGTTTTCTTTGC

TTTAAGTGTAACTGGCAGTTTTCCATTGGTTTACCTGTGAAATAGTTCAAAGCCAAGTTT

ATATACAATTATATCAGTCCTCTTTCAAAGGTAGCCATCATGGATCTGGTAGGGGGAAAA
```

Fig 2e

Session Name: rb

TGTGTATTTTATTACATCTTTCACATTGGCTATTTAAAGACAAAGACAAATTCTGTTTCT

TGAGAAGAGAATATTAGCTTTACTGTTTGTTATGGCTTAATGACACTAGCTAATATCAAT

AGAAGGATGTACATTTCCAAATTCACAAGTTGTGTTTGATATCCAAAGCTGAATACATTC

TGCTTTCATCTTGGTCACATACAATTATTTTTACAGTTCTCCCAAGGGAGTTAGGCTATT

CACAACCACTCATTCAAAAGTTGAAATTAACCATAGATGTAGATAAACTCAGAAATTTAA

TTCATGTTTCTTAAATGGGCTACTTTGTCCTTTTTGTTATTAGGGTGGTATTTAGTCTAT

TAGCCACAAAATTGGGAAAGGAGTAGAAAAAGCAGTAACTGACAACTTGAATAATACACC

AGAGATAATATGAGAATCAGATCATTTCAAAACTCATTTCCTATGTAACTGCATTGAGAA

CTGCATATGTTTCGCTGATATATGTGTTTTTCACATTTGCGAATGGTTCCATTCTCTCTC

CTGTACTTTTTCCAGACACTTTTTTGAGTGGATGATGTTTCGTGAAGTATACTGTATTTT

TACCTTTTTCCTTCCTTATCACTGACACAAAAAGTAGATTAAGAGATGGGTTTGACAAGG

TTCTTCCCTTTTACATACTGCTGTCTATGTGGCTGTATCTTGTTTTTCCACTACTGCTAC

CACAACTATATTATCATGCAAATGCTGTATTCTTCTTTGGTGGAGATAAAGATTTCTTGA

GTTTTGTTTTAAAATTAAAGCTAAAGTATCTGTATTGCATTAAATATAATATCGACACAG

TGCTTTCCGTGGCACTGCATACAATCTGAGGCCTCCTCTCTCAGTTTTTATATAGATGGC

GAGAACCTAAGTTTCAGTTGATTTTACAATTGAAATGACTAAAAAACAAAGAAGACAACA

TTAAAAACAATATTGTTTCTAAAAAAAAAAAAAAAAAAA

Translated Mol. Weight = 86058.72

FIG 3a

GCCGAGCCCCTCCGCAGACTCTGCGCCGGAAAGTTTCATTTGCTGTATGCCATCCTCGA

GAGCTGTCTAGGTTAACGTTCGCACTCTGTGTATATAACCTCGACAGTCTTGGCACCTA

ACGTGCTGTGCGTAGCTGCTCCTTTGGTTGAATCCCCAGGCCCTTGTTGGGGCACAAGG

1 10
met ser gln trp tyr glu leu gln gln leu asp ser lys
TGGCAGG ATG TCT CAG TGG TAC GAA CTT CAG CAG CTT GAC TCA AAA

20
phe leu glu gln val his gln leu tyr asp asp ser phe pro met
TTC CTG GAG CAG GTT CAC CAG CTT TAT GAT GAC AGT TTT CCC ATG

30 40
glu ile arg gln tyr leu ala gln trp leu glu lys gln asp trp
GAA ATC AGA CAG TAC CTG GCA CAG TGG TTA GAA AAG CAA GAC TGG

50
glu his ala ala asn asp val ser phe ala thr ile arg phe his
GAG CAC GCT GCC AAT GAT GTT TCA TTT GCC ACC ATC CGT TTT CAT

60 70
asp leu leu ser gln leu asp asp gln tyr ser arg phe ser leu
GAC CTC CTG TCA CAG CTG GAT GAT CAA TAT AGT CGC TTT TCT TTG

80
glu asn asn phe leu leu gln his asn ile arg lys ser lys arg
GAG AAT AAC TTC TTG CTA CAG CAT AAC ATA AGG AAA AGC AAG CGT

90 100
asn leu gln asp asn phe gln glu asp pro ile gln met ser met
AAT CTT CAG GAT AAT TTT CAG GAA GAC CCA ATC CAG ATG TCT ATG

110
ile ile tyr ser cys leu lys glu glu arg lys ile leu glu asn
ATC ATT TAC AGC TGT CTG AAG GAA GAA AGG AAA ATT CTG GAA AAC

120 130
ala gln arg phe asn gln ala gln ser gly asn ile gln ser thr
GCC CAG AGA TTT AAT CAG GCT CAG TCG GGG AAT ATT CAG AGC ACA

140
val met leu asp lys gln lys glu leu asp ser lys val arg asn
GTG ATG TTA GAC AAA CAG AAA GAG CTT GAC AGT AAA GTC AGA AAT

150 160
val lys asp lys val met cys ile glu his glu ile lys ser leu
GTG AAG GAC AAG GTT ATG TGT ATA GAG CAT GAA ATC AAG AGC CTG

170
glu asp leu gln asp glu tyr asp phe lys cys lys thr leu gln

FIG 3b

Session Name: rb

```
GAA GAT TTA CAA GAT GAA TAT GAC TTC AAA TGC AAA ACC TTG CAG

    180                                                 190
asn arg glu his glu thr asn gly val ala lys ser asp gln lys
AAC AGA GAA CAC GAG ACC AAT GGT GTG GCA AAG AGT GAT CAG AAA

                        200
gln glu gln leu leu leu lys lys met tyr leu met leu asp asn
CAA GAA CAG CTG TTA CTC AAG AAG ATG TAT TTA ATG CTT GAC AAT

    210                                                 220
lys arg lys glu val val his lys ile ile glu leu leu asn val
AAG AGA AAG GAA GTA GTT CAC AAA ATA ATA GAG TTG CTG AAT GTC

                        230
thr glu leu thr gln asn ala leu ile asn asp glu leu val glu
ACT GAA CTT ACC CAG AAT GCC CTG ATT AAT GAT GAA CTA GTG GAG

    240                                                 250
trp lys arg arg gln gln ser ala cys ile gly gly pro pro asn
TGG AAG CGG AGA CAG CAG AGC GCC TGT ATT GGG GGG CCG CCC AAT

                        260
ala cys leu asp gln leu gln gln val arg gln gln leu lys lys
GCT TGC TTG GAT CAG CTG CAG CAA GTT CGG CAG CAG CTT AAA AAG

    270                                                 280
leu glu glu leu glu gln lys tyr thr tyr glu his asp pro ile
TTG GAG GAA TTG GAA CAG AAA TAC ACC TAC GAA CAT GAC CCT ATC

                        290
thr lys asn lys gln val leu trp asp arg thr phe ser leu phe
ACA AAA AAC AAA CAA GTG TTA TGG GAC CGC ACC TTC AGT CTT TTC

    300                                                 310
gln gln leu ile gln ser ser phe val val glu arg gln pro cys
CAG CAG CTC ATT CAG AGC TCG TTT GTG GTG GAA AGA CAG CCC TGC

                        320
met pro thr his pro gln arg pro leu val leu lys thr gly val
ATG CCA ACG CAC CCT CAG AGG CCG CTG GTC TTG AAG ACA GGG GTC

    330                                                 340
gln phe thr val lys leu arg leu leu val lys leu gln glu leu
CAG TTC ACT GTG AAG TTG AGA CTG TTG GTG AAA TTG CAA GAG CTG

                        350
asn tyr asn leu lys val lys val leu phe asp lys asp val asn
AAT TAT AAT TTG AAA GTC AAA GTC TTA TTT GAT AAA GAT GTG AAT

    360                                                 370
glu arg asn thr val lys gly phe arg lys phe asn ile leu gly
GAG AGA AAT ACA GTA AAA GGA TTT AGG AAG TTC AAC ATT TTG GGC

                        380
thr his thr lys val met asn met glu glu ser thr asn gly ser
ACG CAC ACA AAA GTG ATG AAC ATG GAG GAG TCC ACC AAT GGC AGT
```

FIG 3c

Session Name: rb

```
    390                                                 400
leu ala ala glu phe arg his leu gln leu lys glu gln lys asn
CTG GCG GCT GAA TTT CGG CAC CTG CAA TTG AAA GAA CAG AAA AAT

                        410
ala gly thr arg thr asn glu gly pro leu ile val thr glu glu
GCT GGC ACC AGA ACG AAT GAG GGT CCT CTC ATC GTT ACT GAA GAG

    420                                                 430
leu his ser leu ser phe glu thr gln leu cys gln pro gly leu
CTT CAC TCC CTT AGT TTT GAA ACC CAA TTG TGC CAG CCT GGT TTG

                        440
val ile asp leu glu thr thr ser leu pro val val val ile ser
GTA ATT GAC CTC GAG ACG ACC TCT CTG CCC GTT GTG GTG ATC TCC

    450                                                 460
asn val ser gln leu pro ser gly trp ala ser ile leu trp tyr
AAC GTC AGC CAG CTC CCG AGC GGT TGG GCC TCC ATC CTT TGG TAC

                        470
asn met leu val ala glu pro arg asn leu ser phe phe leu thr
AAC ATG CTG GTG GCG GAA CCC AGG AAT CTG TCC TTC TTC CTG ACT

    480                                                 490
pro pro cys ala arg trp ala gln leu ser glu val leu ser trp
CCA CCA TGT GCA CGA TGG GCT CAG CTT TCA GAA GTG CTG AGT TGG

                        500
gln phe ser ser val thr lys arg gly leu asn val asp gln leu
CAG TTT TCT TCT GTC ACC AAA AGA GGT CTC AAT GTG GAC CAG CTG

    510                                                 520
asn met leu gly glu lys leu leu gly pro asn ala ser pro asp
AAC ATG TTG GGA GAG AAG CTT CTT GGT CCT AAC GCC AGC CCC GAT

                        530
gly leu ile pro trp thr arg phe cys lys glu asn ile asn asp
GGT CTC ATT CCG TGG ACG AGG TTT TGT AAG GAA AAT ATA AAT GAT

    540                                                 550
lys asn phe pro phe trp leu trp ile glu ser ile leu glu leu
AAA AAT TTT CCC TTC TGG CTT TGG ATT GAA AGC ATC CTA GAA CTC

                        560
ile lys lys his leu leu pro leu trp asn asp gly cys ile met
ATT AAA AAA CAC CTG CTC CCT CTC TGG AAT GAT GGG TGC ATC ATG

    570                                                 580
gly phe ile ser lys glu arg glu arg ala leu leu lys asp gln
GGC TTC ATC AGC AAG GAG CGA GAG CGT GCC CTG TTG AAG GAC CAG

                        590
gln pro gly thr phe leu leu arg phe ser glu ser ser arg glu
CAG CCG GGG ACC TTC CTG CTG CGG TTC AGT GAG AGC TCC CGG GAA
```

FIG 3d

Session Name: rb

```
                                                    610
    600
gly ala ile thr phe thr trp val glu arg ser gln asn gly gly
GGG GCC ATC ACA TTC ACA TGG GTG GAG CGG TCC CAG AAC GGA GGC

                        620
glu pro asp phe his ala val glu pro tyr thr lys lys glu leu
GAA CCT GAC TTC CAT GCG GTT GAA CCC TAC ACG AAG AAA GAA CTT

                                                    640
    630
ser ala val thr phe pro asp ile ile arg asn tyr lys val met
TCT GCT GTT ACT TTC CCT GAC ATC ATT CGC AAT TAC AAA GTC ATG

                        650
ala ala glu asn ile pro glu asn pro leu lys tyr leu tyr pro
GCT GCT GAG AAT ATT CCT GAG AAT CCC CTG AAG TAT CTG TAT CCA

                                                    670
    660
asn ile asp lys asp his ala phe gly lys tyr tyr ser arg pro
AAT ATT GAC AAA GAC CAT GCC TTT GGA AAG TAT TAC TCC AGG CCA

                        680
lys glu ala pro glu pro met glu leu asp gly pro lys gly thr
AAG GAA GCA CCA GAG CCA ATG GAA CTT GAT GGC CCT AAA GGA ACT

                                                700 701
    690
gly tyr ile lys thr glu leu ile ser val ser glu val OC
GGA TAT ATC AAG ACT GAG TTG ATT TCT GTG TCT GAA GTG TAA GTGAAC

ACAGAAGAGTGACATGTTTACAAACCTCAAGCCAGCCTTGCTCCTGGCTGGGGCCTGTTG

AAGATGCTTGTATTTTACTTTTCCATTGTAATTGCTATCGCCATCACAGCTGAACTTGTT

GAGATCCCCGTGTTACTGCCTATCAGCATTTTACTACTTTAAAAAAAAAAAAAAAAGCCA

AAAACCAAATTTGTATTTAAGGTATATAAATTTTCCCAAAACTGATACCCTTTGAAAAAG

TATAAATAAAATGAGCAAAAGTTGAAAAAAAAAAAAAAAAAAA

Translated Mol. Weight = 81766.23
```

FIG 4

The Polypeptides of ISGF-3

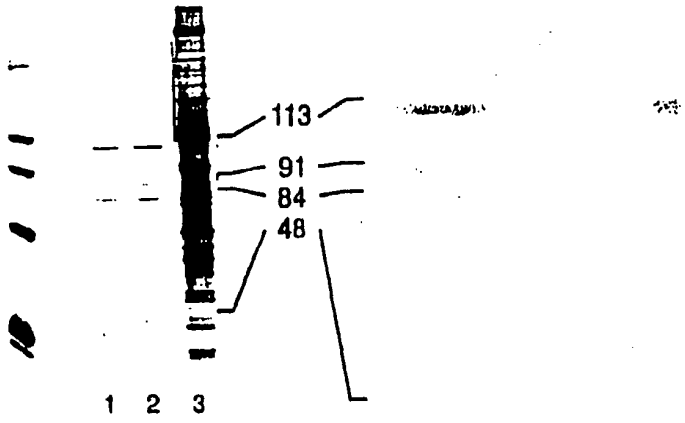


FIG 5

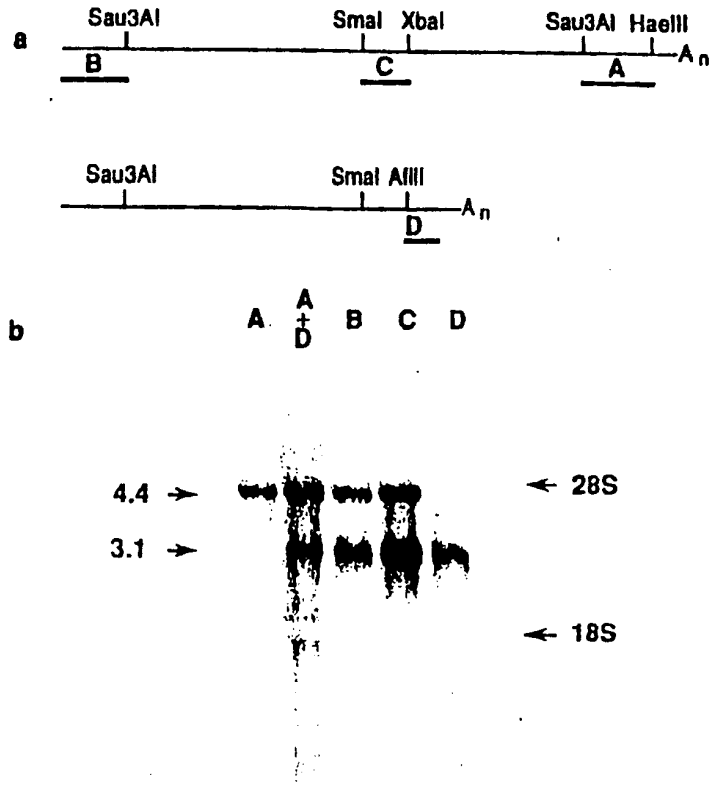


FIG 6

Amino Acid Sequence of the 91 kd and 84 kd Proteins

```
  1 MSQWYELQQLDSKFLEQVHQLYDDSFPMEIRQYLAQWLEKQDWEHAANDV
 51 SFATIRFHDLLSQLDDQYSRFSLENNFLLQHNIRKSKRNLQDNFQEDPIQ
101 MSMIIYSCLKEERKILENAQRFNQAQSGNIQSTVMLDKQKELDSKVRNVK
151 DKVMCIEHEIKSLEDLQDEYDFKCKTLQNREHETNGVAKSDQKQEQLLLK
201 KMYLMLDNKRKEVVHKIIELLNVTELTQNALINDELVEWKRRQQSACIGG
251 PPNACLDQLQQVRQQLKKLEELEQKYTYEHDPITKNKQVLWDRTFSLFQQ
301 LIQSSFVVERQPCMPTHPQRPLVLKTGVQFTVKLRLLVKLQELNYNLKVK
351 VLFDKDVNERNTVKGFRKFNILGTH[illegible]KVMNMEESTNGSLAAEFRHLQLKE
401 QKNAGTRTNEGPLIVTEELHSLSFETQLCQPGLVIDLETTSLPVVVISNV
451 SQLPSGWASILWYNMLVAEPRNLSFFLTPPCARWAQLSEVLSWQFSSVTK
                 t27
501 RGLNVDQLNMLGEKLLGPNASPDGLIPWTRFCKENINDKNFPFWLWIESI
                 t19
551 LELIKKHLLPLWNDGCIMGFISKERERALLKDQQPGTFLLRFSESSREGA
601 ITFTWVERSQNGGEPDFHAVEPYTKKELSAVTFPDIIRNYKVMAAENIPE
                                                    t13a
651 NPLKYLYPNIDKDHAFGKYYSRPKEAPEPMELDGPKGTGYIKTELISVSE
                 t13b
701 VHPSRLQTTDNLLPMSPEEFDEVSRIVGSVEFDSMMNTV
    ▲
last amino acid of 84 kd
```

Fig 7a

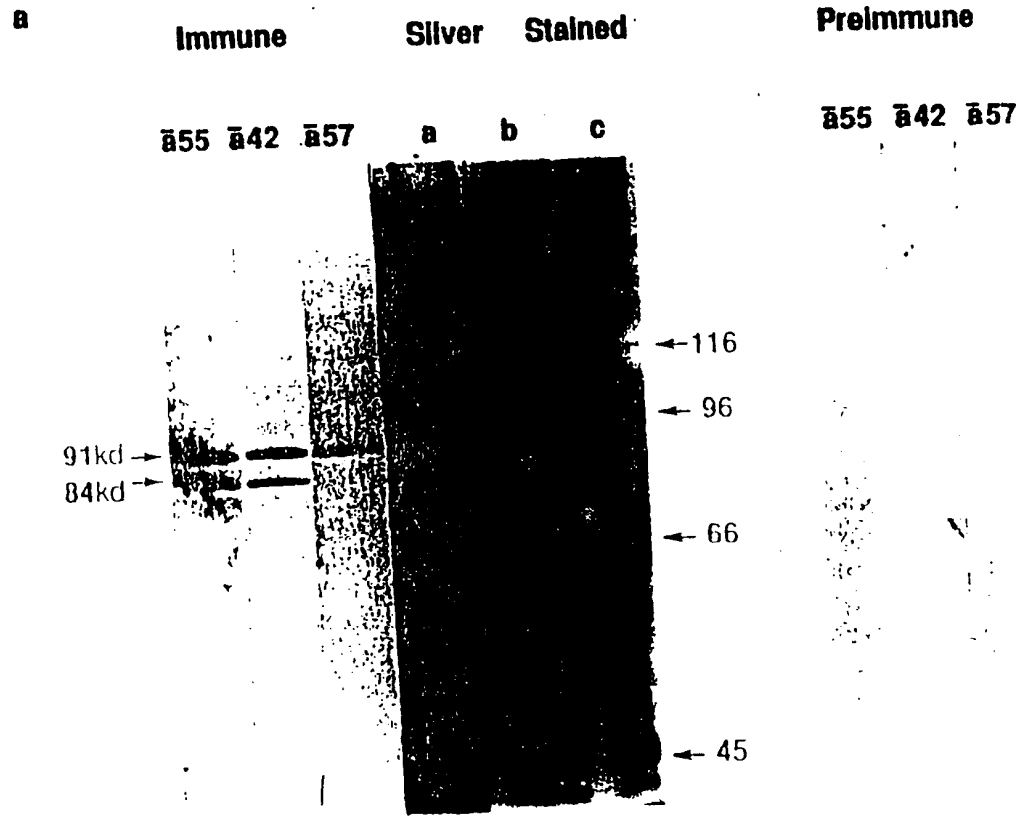

a
Immune
Silver Stained
Preimmune
ā55 ā42 ā57
a b c
ā55 ā42 ā57
91kd
84kd
116
96
66
45

FIG 8Tb

b

a b c d e

ISGF-3 →

γ-Component →

FIG. 8a

A.

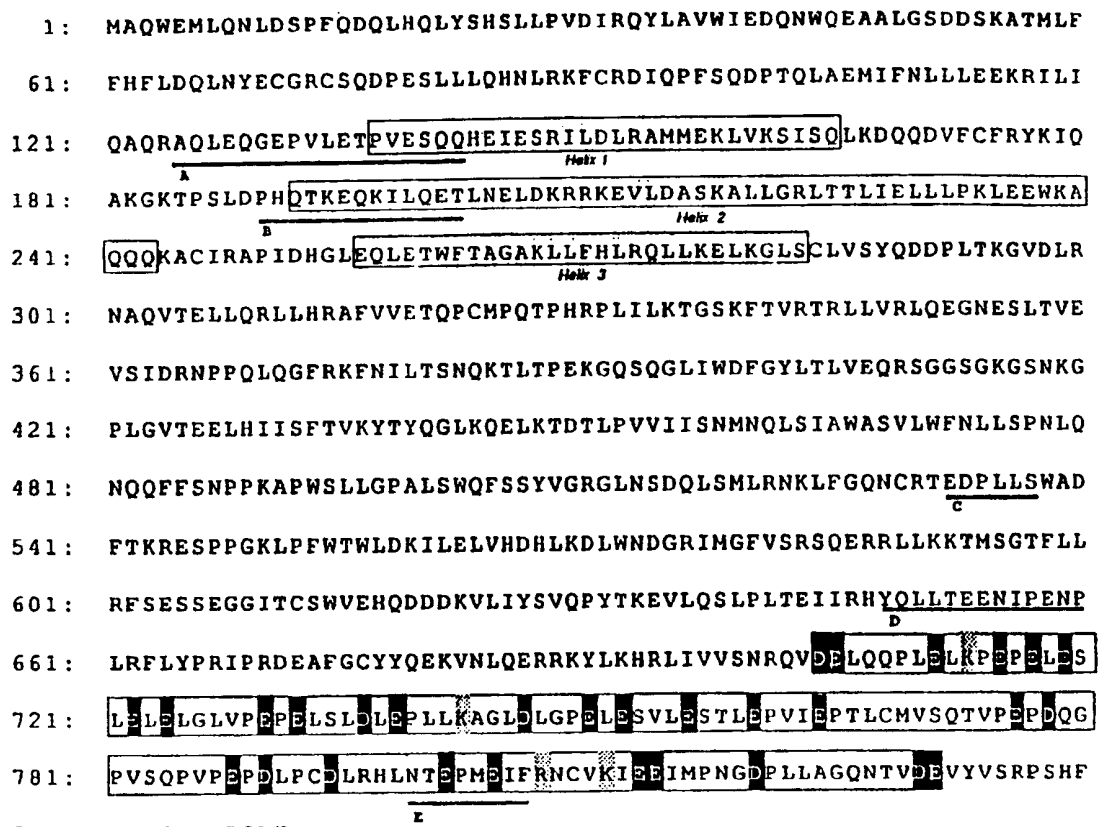

1: MAQWEMLQNLDSPFQDQLHQLYSHSLLPVDIRQYLAVWIEDQNWQEAALGSDDSKATMLF
61: FHFLDQLNYECGRCSQDPESLLLQHNLRKFCRDIQPFSQDPTQLAEMIFNLLLEEKRILI
121: QAQRAQLEQGEPVLETPVESQQHEIESRILDLRAMMEKLVKSISQLKDQQDVFCFRYKIQ
Helix 1
181: AKGKTPSLDPHQTKEQKILQETLNELDKRRKEVLDASKALLGRLTTLIELLLPKLEEWKA
Helix 2
241: QQQKACIRAPIDHGLEQLETWFTAGAKLLFHLRQLLKELKGLSCLVSYQDDPLTKGVDLR
Helix 3
301: NAQVTELLQRLLHRAFVVETQPCMPQTPHRPLILKTGSKFTVRTRLLVRLQEGNESLTVE
361: VSIDRNPPQLQGFRKFNILTSNQKTLTPEKGQSQGLIWDFGYLTLVEQRSGGSGKGSNKG
421: PLGVTEELHIISFTVKYTYQGLKQELKTDTLPVVIISNMNQLSIAWASVLWFNLLSPNLQ
481: NQQFFSNPPKAPWSLLGPALSWQFSSYVGRGLNSDQLSMLRNKLFGQNCRTEDPLLSWAD
541: FTKRESPPGKLPFWTWLDKILELVHDHLKDLWNDGRIMGFVSRSQERRLLKKTMSGTFLL
601: RFSESSEGGITCSWVEHQDDDKVLIYSVQPYTKEVLQSLPLTEIIRHYQLLTEENIPENP
661: LRFLYPRIPRDEAFGCYYQEKVNLQERRKYLKHRLIVVSNRQVDELQQPLELKPEPELDS
721: LELELGLVPEPELSLDLEPLLKAGLDLGPELESVLESTLEPVIEPTLCMVSQTVPEPDQG
781: PVSQPVPEPDLPCDLRHLNTEPMEIFRNCVKIEEIMPNGDPLLAGQNTVDEVYVSRPSHF
841: YTDGPLMPSDF

FIG 8b

B.

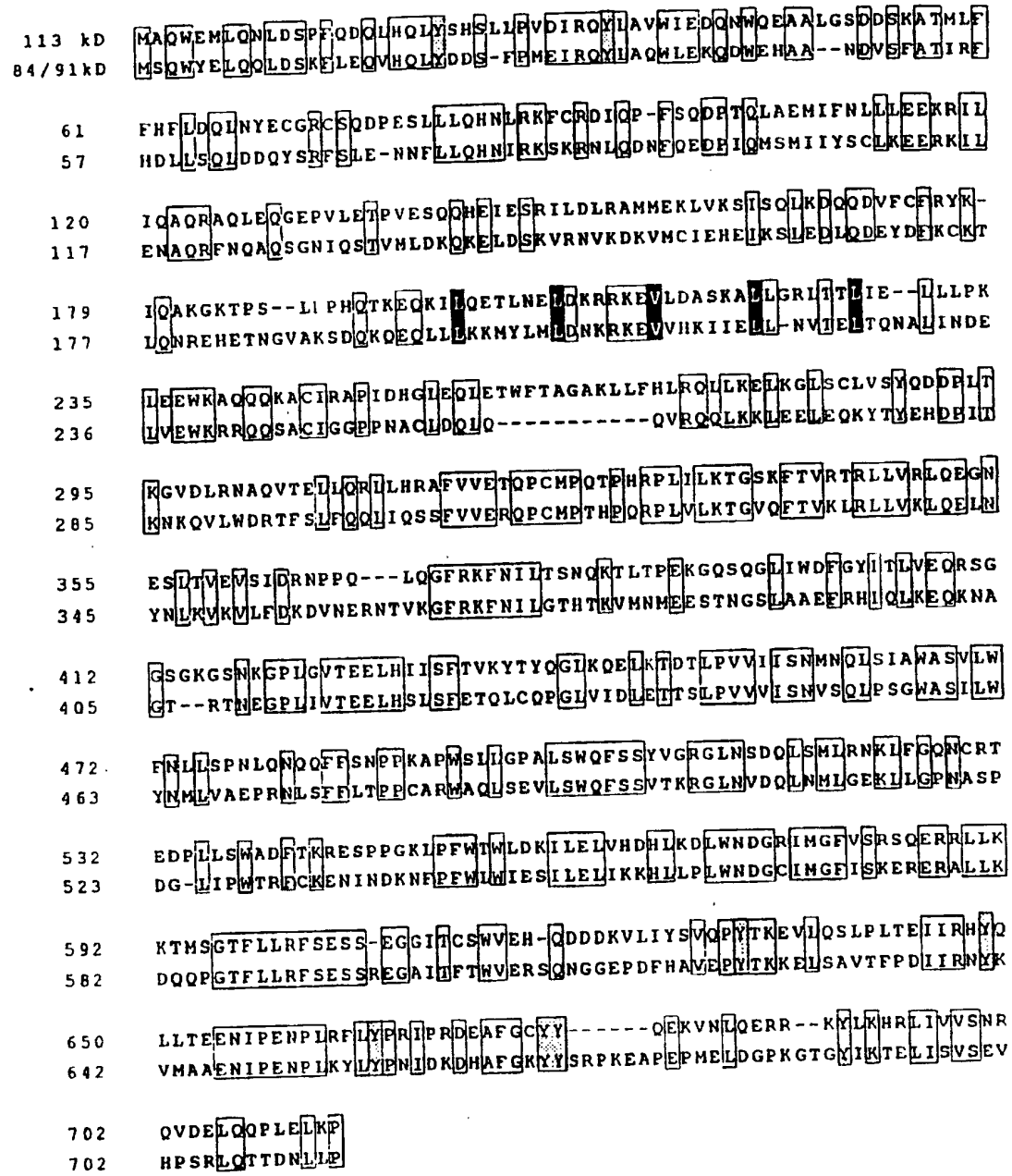
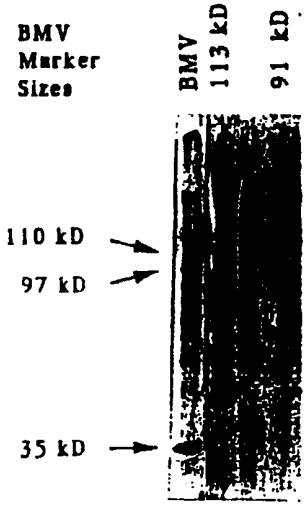

113 kD
84/91kD
61
57
120
117
179
177
235
236
295
285
355
345
412
405
472
463
532
523
592
582
650
642
702
702

FIG 9

A.



B.

FIG 10

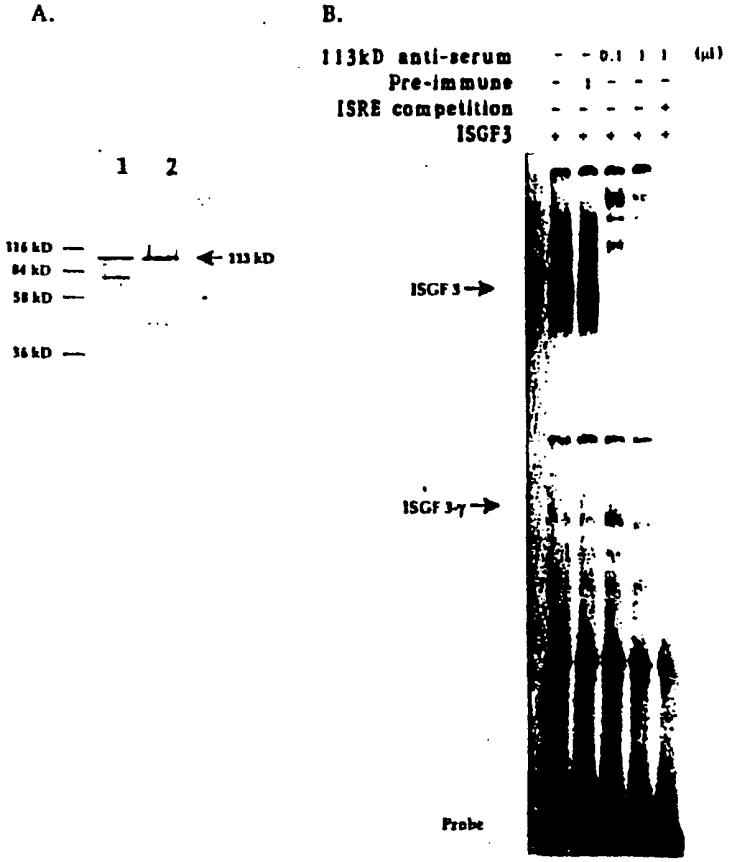

A.
1 2
116 kD
84 kD
58 kD
36 kD
113 kD
B.
113kD anti-serum - - 0.1 1 1 (µl)
Pre-immune - 1 - - -
ISRE competition - - - - +
ISGF3 + + + + +
ISGF 3
ISGF 3-γ
Probe

Fig. 3

FIG 11

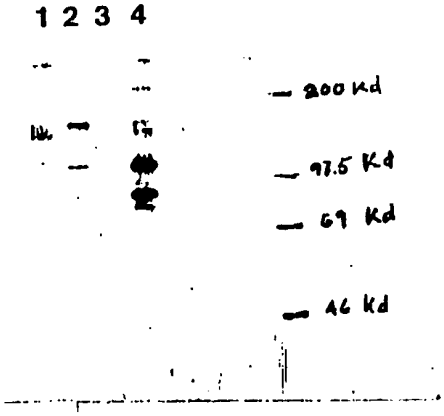


FIG. 12

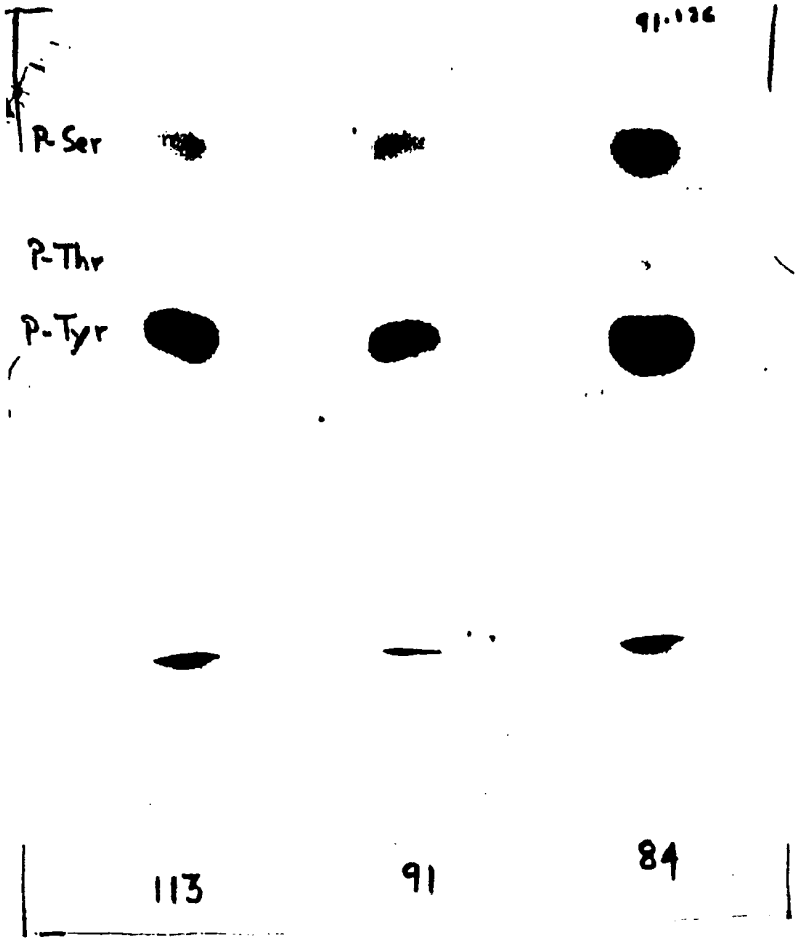


FIGURE 13

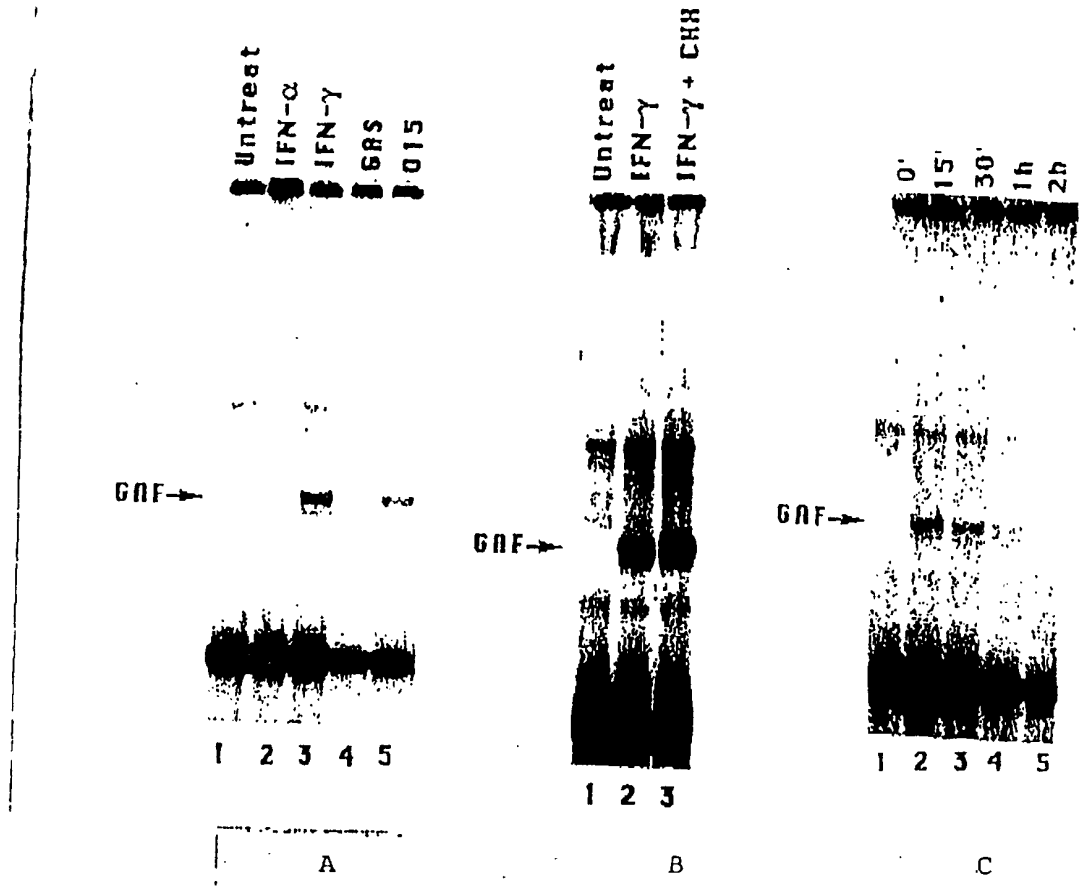


FIGURE 14

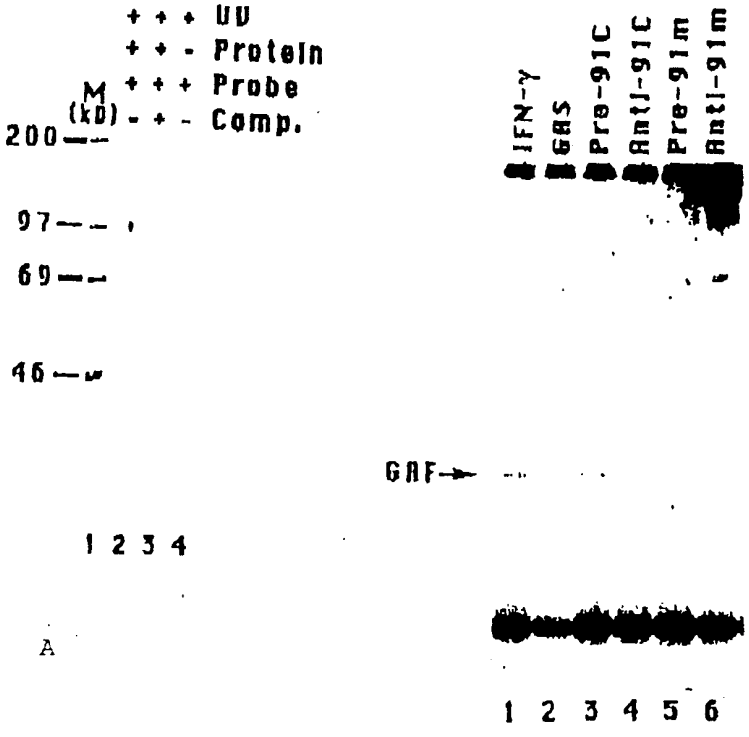


FIGURE 14- CONT'D.

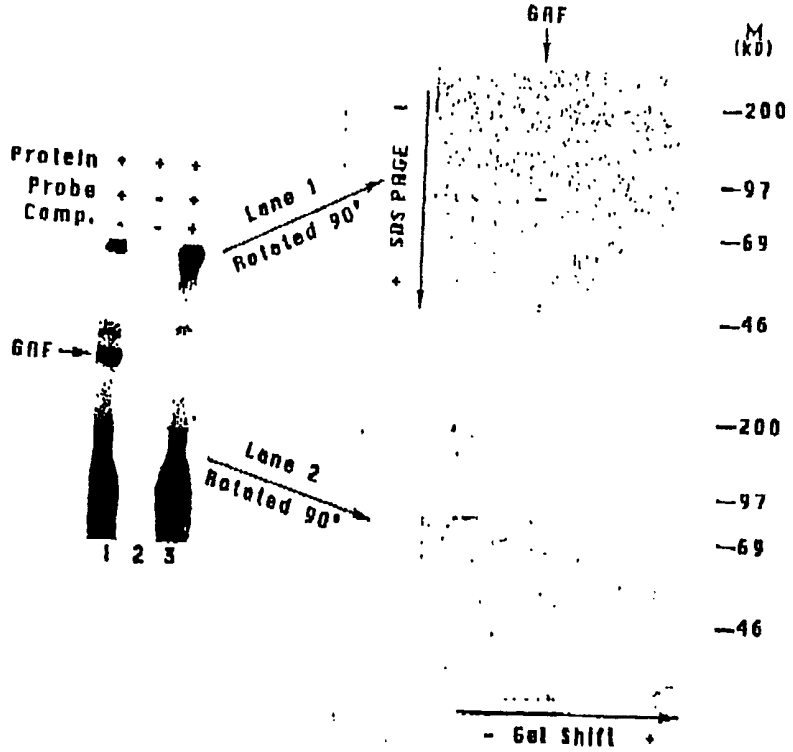


FIG. 14C

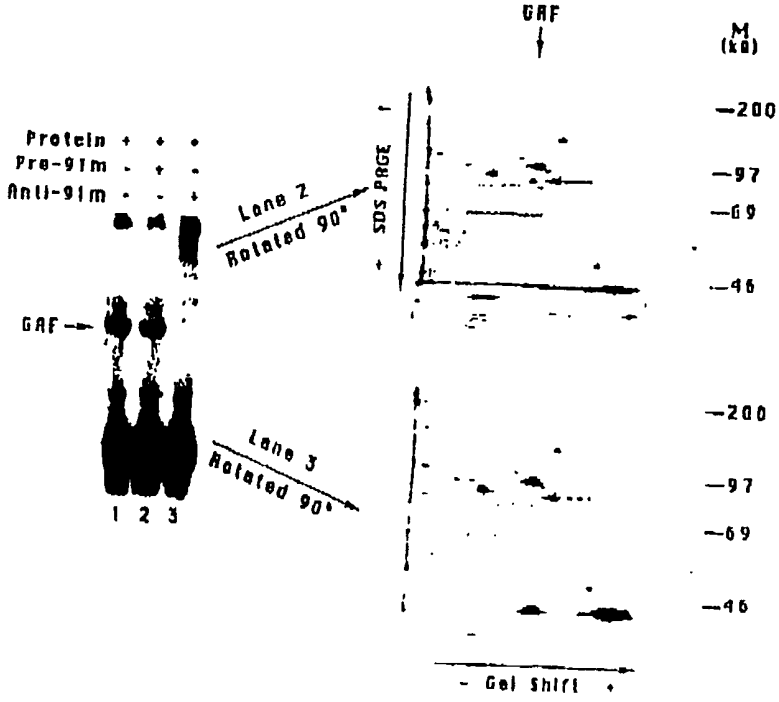


FIG. 14D

FIGURE 15

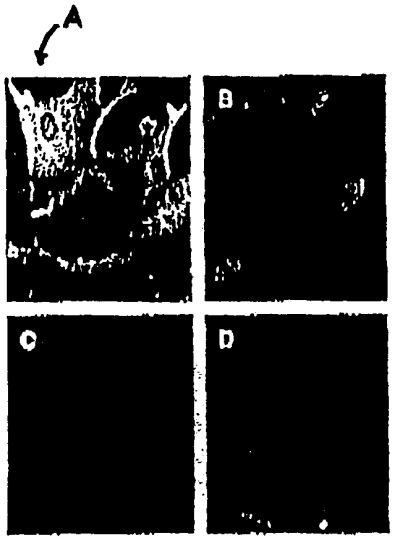
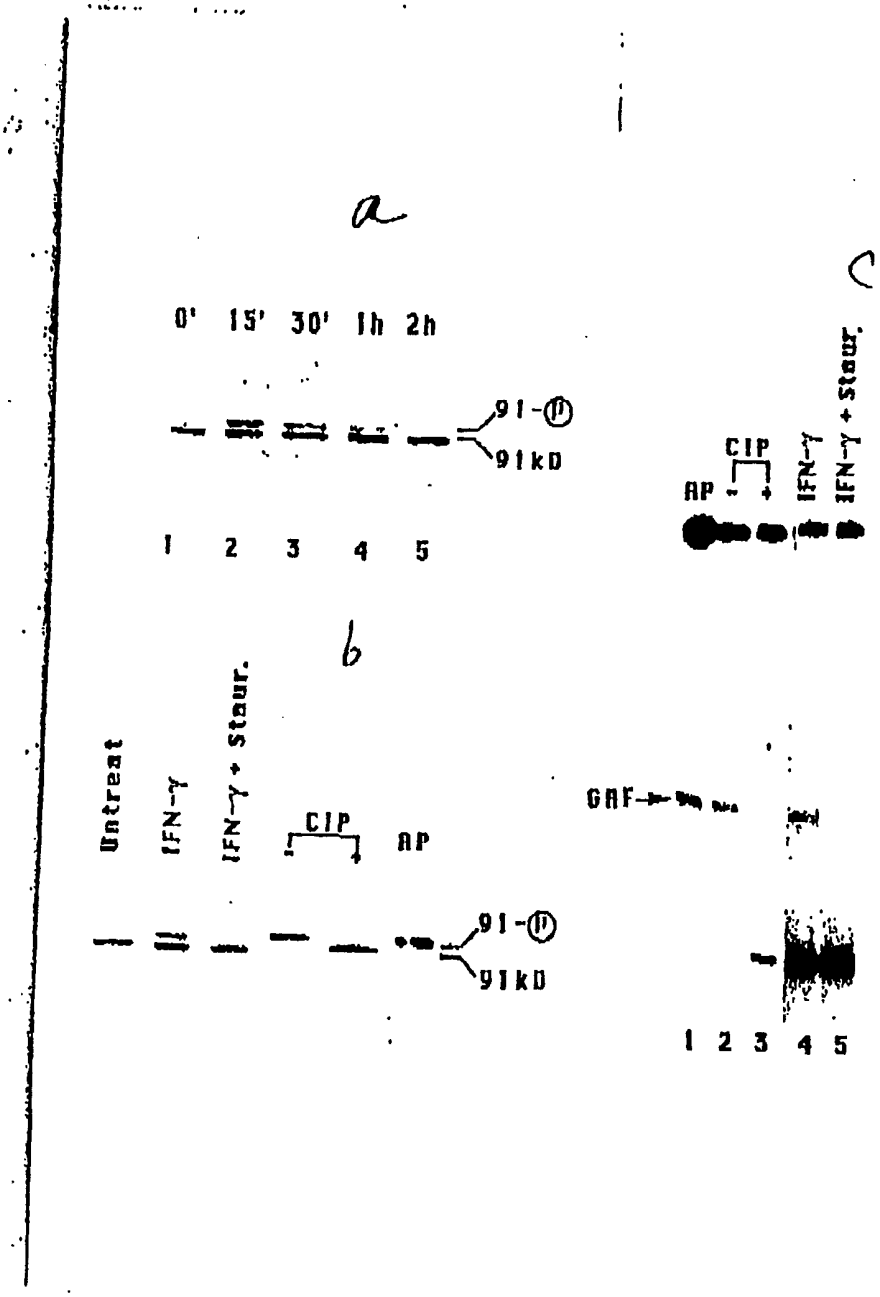


FIGURE 16


a
0' 15' 30' 1h 2h
91-(II)
91kD
1 2 3 4 5
b
Untreat
IFN-γ
IFN-γ + Staur.
CIP
AP
91-(II)
91kD
c
CIP
AP
IFN-γ
IFN-γ + Staur.
GAF
1 2 3 4 5

a

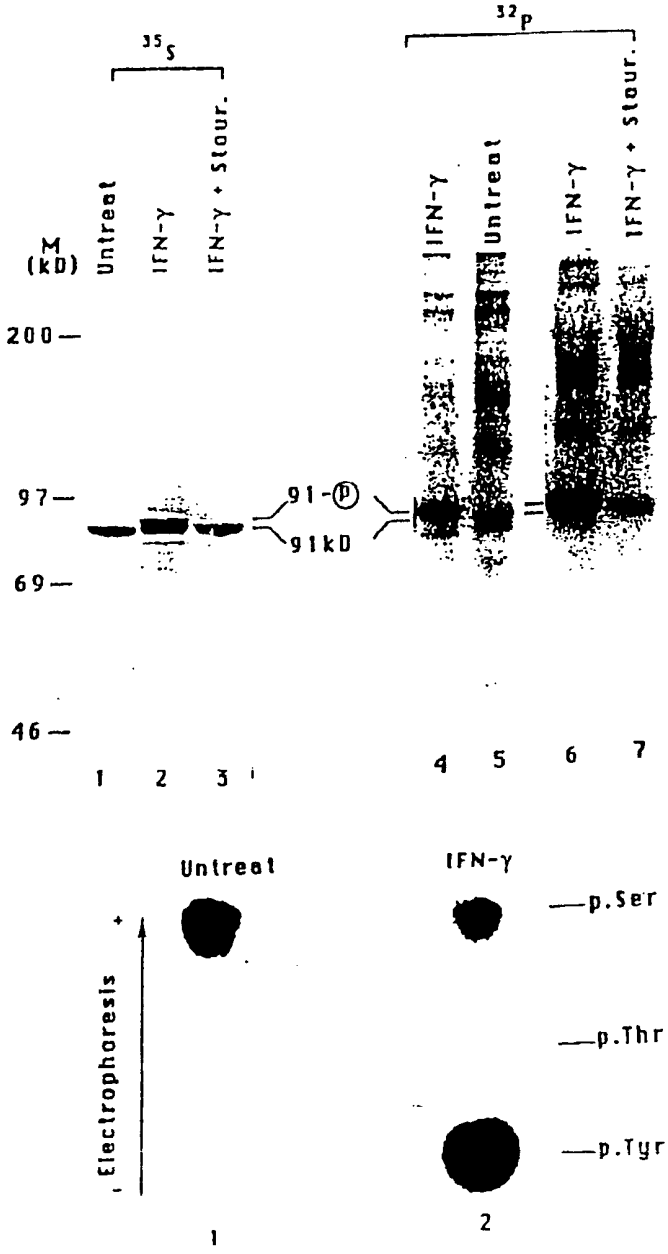
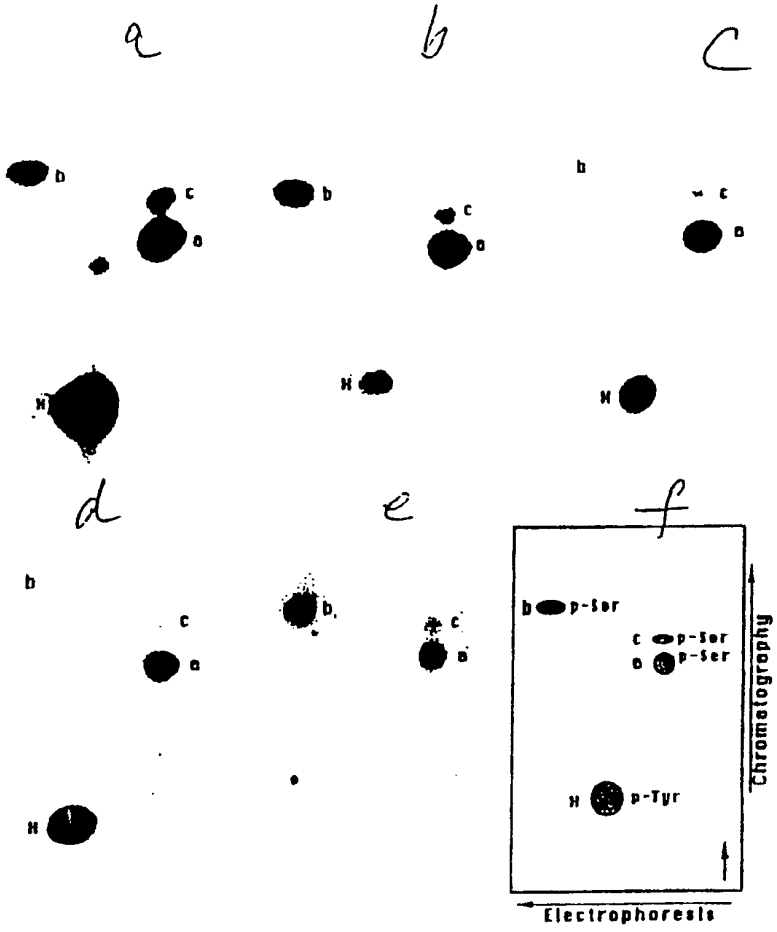

35S
32P
M (kD)
Untreat
IFN-γ
IFN-γ + Staur.
IFN-γ
Untreat
IFN-γ
IFN-γ + Staur.
200
97
69
46
91-Ⓟ
91kD
1 2 3 4 5 6 7
b
Untreat
IFN-γ
p.Ser
p.Thr
p.Tyr
Electrophoresis
+
-
1
2

FIGURE 17

FIGURE 18


a
b
c
d
e
f
b
c
a
H
b p-Ser
c p-Ser
a p-Ser
H p-Tyr
Chromatography
Electrophoresis

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/02569

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/12; C07K13/00; C12P21/08; C12N15/11
C12N9/00; C12N1/21; C12N5/10; G01N33/68

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C07K ; G01N ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 87, no. 21, November 1990, WASHINGTON US pages 8555 - 8559 X.-Y. FU ET AL.; 'ISGF3, the transcriptional activator induced by interferon alpha, consists of multiple interacting polypeptide chains' cited in the application see the whole document	1-8, 17-23, 53-55, 60,61,74
Y	cited in the application	75-87

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
16 AUGUST 1993	27 -08- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	NAUCHE S.A.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category°	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	THE NEW BIOLOGIST vol. 2, no. 10, October 1990, PHILADELPHIA, US pages 923 - 928 D. LEVY ET AL.; 'Interferon-dependent transcriptional activation: Signal transduction without second messenger involvement?' cited in the application	1--23, 53-60, 61,74
Y	see the whole document cited in the application	75-87
X	GENES AND DEVELOPMENT vol. 4, no. 10, October 1990, COLD SPRING HARBOR, NY, US pages 1753 - 1765 D.S. KESSLER ET AL.; 'Interferon-alpha regulates nuclear translocation and DNA-binding affinity of ISGF3; a multimeric transcriptional activator' cited in the application	1-8, 17-23, 53-55, 60,61,74
Y	see the whole document cited in the application	75-87
Y	GENE. vol. 72, 1988, AMSTERDAM NL pages 25 - 34 MASAYORI INOUYE 'Antisense RNA : its funcions and applications in gene regulation - a review' see page 29, column 1, line 7 - page 32	75-81
Y	TRENDS IN BIOTECHNOLOGY vol. 8, no. 7, July 1990, CAMBRIDGE GB pages 174 - 178 MATT COTTEN 'The in vivo application of ribozymes' see the whole document	82,84-87
Y	BIOTECHNOLOGY vol. 10, March 1992, NEW YORK US pages 256 - 262 EDGINGTON, S.M. 'Ribozymes : Stop making sense' see the whole document	82-87

-/--

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	EMBO JOURNAL. vol. 10, no. 4, April 1991, EYNSHAM, OXFORD GB pages 927 - 932 DECKER T;LEW DJ;MIRKOVITCH J;DARNELL JE JR; 'Cytoplasmic activation of GAF, an IFN-gamma-regulated DNA-binding factor.' cited in the application see the whole document	1-87
A	MOLECULAR AND CELLULAR BIOLOGY vol. 11, no. 10, October 1991, WASHINGTON US pages 5147 - 5133 DECKER T;LEW DJ;DARNELL JE JR; 'Two distinct alpha-interferon-dependent signal transduction pathways may contribute to activation of transcription of the guanylate-binding protein gene.'	
P,X	WO,A,9 208 740 (THE ROCKEFELLER UNIVERSITY, US) 29 May 1992 cited in the application see the whole document	1-74
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 89, no. 16, 15 August 1992, WASHINGTON US pages 7836 - 7839 SCHINDLER C;FU XY;IMPROTA T;AEBERSOLD R;DARNELL JE JR; 'Proteins of transcription factor ISGF-3: one gene encodes the 91-and 84-kDa ISGF-3 proteins that are activated by interferon alpha.'	1-8, 17-23, 53-55, 60,61,74
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 89, no. 16, 15 August 1992, WASHINGTON US pages 7840 - 7843 FU XY; SCHINDLER C;IMPROTA T;AEBERSOLD R;DARNELL JE JR; 'The proteins of ISGF-3, the interferon alpha-induced transcriptional activator, define a gene family involved in signal transduction.' see the whole document	1-8, 17-23, 53-55, 60,61,74

-/--

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	SCIENCE vol. 258, 11 December 1992, LANCASTER, PA pages 1808 - 1812 SHUAI, K. ET AL.; 'Activation of transcription by IFN-gamma: Tyrosine phosphorylation of a 91-kD DNA binding protein.' see the whole document	1-8, 17-23, 53-55, 60,61,74

INTERNATIONAL SEARCH REPORT

Int ional application No.
PCT/US93/02569

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 42-49, 62-68, 70-73 are directed to a method of treatment of the human/animal body (Rule 39.1(iv) PCT) the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9302569
SA 72001

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 16/08/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9208740	29-05-92	AU-A- 9092091	11-06-92

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

HPS Trailer Page
for
WEST

UserID: klacourciere

Printer: cm1_11e14_gbegptr

Summary

Document	Pages	Printed	Missed
WO009319179	133	133	0
Total (1)	133	133	0

klacourciere

Printed by HPS Server
for
WEST

Printer: cm1_11e14_gbegptr
Date: 08/16/01
Time: 20:31:53

Document Listing

Document	Selected Pages	Page Range
WO009508629	160	1 - 160
Total (1)	160	-